Proline Suite
User Guide

Proline is a suite of software and components dedicated to mass spectrometry proteomics. Proline lets you extract data from raw files or identification engines, organize and store your data in a relational database, process and analyse this data to finally visualize and extract knowledge from MS based proteomics results.

Proline suite main features

The current version supports the following features:

- **Import identification results** (OMSSA and Mascot files are currently supported). Once imported, search results can then be browsed and visualized through a graphical user interface.
- **Validate search results** using customizable filters and infer proteins identification based on validated PSM. Identification results issued from the validation can obviously be browsed and visualized.

- **Combine individual search results** or identification results to build a comprehensive proteome.
- **Export** identification results in different formats including standard exchange formats.

The software suite is based on three main components:

- A **relational database management system** storing the data used by the software in four different databases
- A **web server** handling processing tasks and web data access
- Two different graphical user interfaces, both allowing users to launch tasks and visualize their data: **Proline Studio** which is a rich client interface and **Proline Web** the web client interface

An additional component is used by administrators to setup and manage Proline (called ProlineAdmin).

Setup and Install

Read the Installation & Setup documentation to install, start the different modules used by Proline or upgrade your installation with a newer version

Getting Started

Discover Proline's workflow and how to execute it with Proline Studio and Proline Web.

How-to

Find quick answer to your questions in this How to section.

Concepts

Read the Concepts & Principles documentation to understand main concepts and algorithms used in Proline.

Releases

Both interfaces, Studio and Web, are based on a set of databases.

Raw file conversion to mzDB

This procedure is detailed in the mzDB Documentation section.

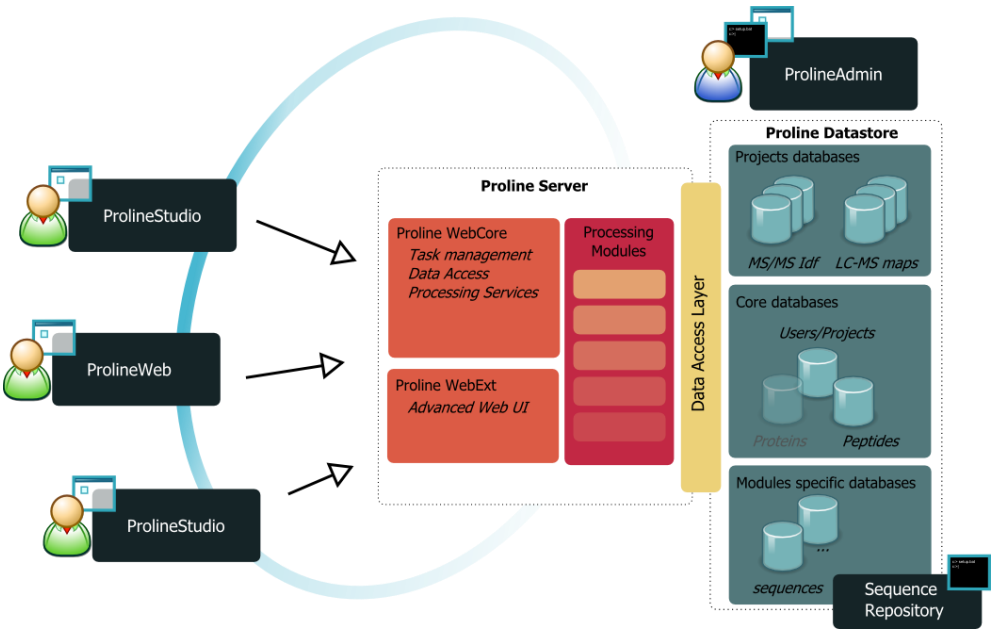
Installation & Setup

This page gives you a short overview of Proline components architecture and explains how to install and setup the different components

Architecture Overview

The suite is based on different components (see figure below):

- A Relational Database Management System (**Proline Datastore**) storing the data used by the software in different databases.
- A web server (**Proline Server**) handling processing tasks and web data access
- Two different graphical user interfaces, both allowing users to launch tasks and visualize their data:
    - ProlineStudio which is a rich client interface
    - ProlineWeb the web client interface
- A daemon application, Proline **Sequence Repository**, automaltically filling proteins sequences repository from fasta.
- A system administration application (**ProlineAdmin**) to setup and manage the Proline suite. This application is available as a command line applicatin or with a graphical user interfaces.



Proline components

Proline DataStore

Proline stores data in six different database schemas. Three of them are “core” database schemas created once at datastore initialization. This three databases contains data related to users projects (**UDS database**), peptides sequences and post-translational modifications (**PS database**) and proteins and databank (**PDI database**). The **Seq database**, where protein ID and sequence are store, is automatically created when running the associated

daemon application (Sequence Repository). This database is needed to have protein sequences and descriptions in user interfaces. The PDI database (with more information than the sequence database) is not available yet.

The two additional schemas are used to create a new database each time you create a new user project. This databases store identification data (**MSI databases**) and quantification data (**LCMS database**) associated with users projects.

Requirements

The server-centric architecture of Proline imposes different requirements for the server computer and the client computers.

- Server-side Proline requirements:
  - a Java SE 8 JRE or above must be installed.
  - the PostgreSQL database server (tested versions are 9.X) must be installed and configured. On windows the automated installer includes the PostgreSQL server, which can be installed on the same computer than Proline or a dinstinct one. By default, PostgreSQL settings are defined for modest hardware configurations but they can be modified to target more efficient hardware configurations (See PostgreSQL optimization).
  - Proline-Server **must run in English "locale"**, on Linux OS, environment variable `LANG=en_US.UTF-8` can be exported before starting ProlineWeb-Core. If not in english, you can also modify the jetty.runner.sh (see installation steps) to add `-Duser.language="en" -Duser.country="US"` parameters
- Client-side requirements for Proline Studio:
  - a Java SE 7 JRE or above must be installed (**on Windows OS, Proline-Studio installer already includes a JRE 32 or 64-bit distribution**)

If you want to use Proline remotely through a Web client, the ProlineWeb components and their requirements must also must installed.

- Server-side ProlineWeb requirements:
  - MongoDB database server must be installed. Note: this database server can be installed on a distinct computer.
- Client-side requirements for Proline Web:
  - a recent Web Browser (IE 9+, Firefox 25+, Chrome 30+).

Installing Proline Suite

- To install Proline for the first time, go here

Installing the Proline Suite

The Proline suite is based on (different components). The following documentation describes the installation procedure for each of this component:

- Proline server
- Sequence Repository
- Proline Studio
- Proline Web

Proline server installation and setup

Proline server installation

Windows users

Download the automated installer from the Proline website (http://proline.profiproteomics.fr/download/).

The wizard will guide you through the installation process.
By default, the installer will unpack all components on the computer. However, it is possible to install the Proline components on distinct computers if it fits better your hardware architecture.

For users who prefer **manual installation or witout administrator rights**, an archive file of the distribution is also available. You can follow the installation procedure described in the next section.

Linux users or manual installation

There is no automated installer at the moment.

First check that all requirements are first installed on the computer.
Then download the zip archive containing Proline components from the dedicated website (http://proline.profiproteomics.fr/download/).

The Proline Server archive file contains three others archives corresponding to the (different components).

- Proline WebCore : the Proline Server
- Proline Admin GUI
- Sequence Repository

Unzip these components on the appropriated computer (Proline Server and Proline Admin is recommanded to be on the same computer. Sequence Repository is recommanded to be installed on the computer where fasta files are accessible)

Once Proline is installed you must initialize Proline datastores and settings.

On this purpose, the Proline-Admin software is provided with the Proline Suite. It is available as a command-line tool or as Graphical Interface called Proline-Admin GUI.

We will guide you through this process, step by step, using both these tools.

Setting up the Datastore

You must first configure ProlineAdmin since this component is used to create the databases needed by Proline.

From graphical tool ProlineAdmin GUI

Launch ProlineAdmin GUI

Windows users

A shortcut “Proline Admin” is available in the Windows Start Menu, under the Proline folder.

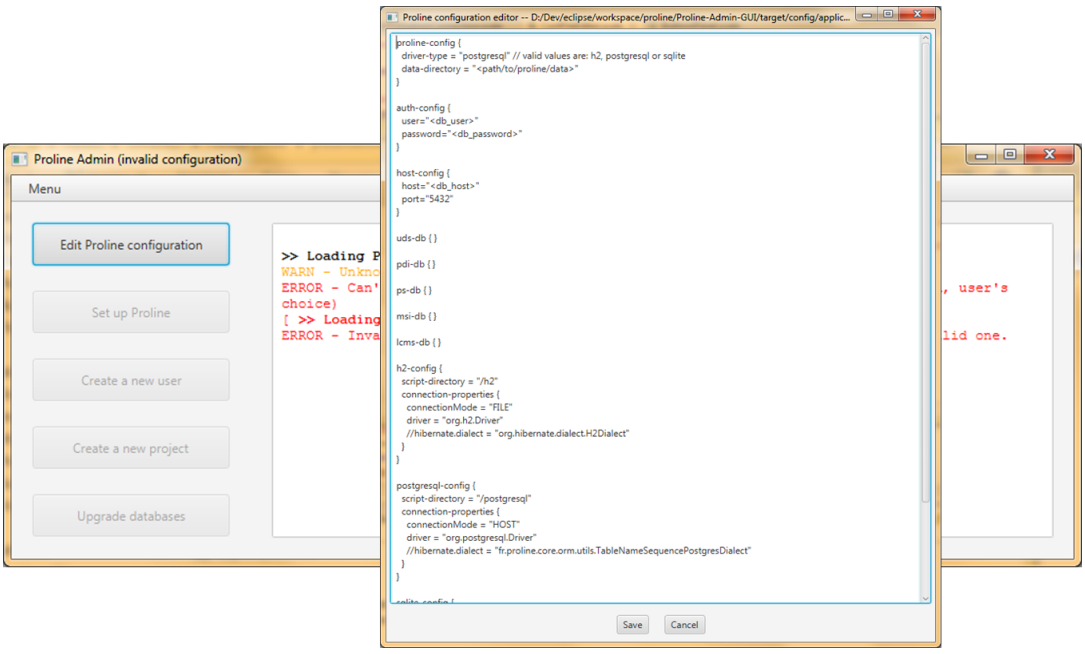
Linux users or manual installation

Execute the `start.sh` script located in the folder obtained after Proline Admin GUI archive file extraction.

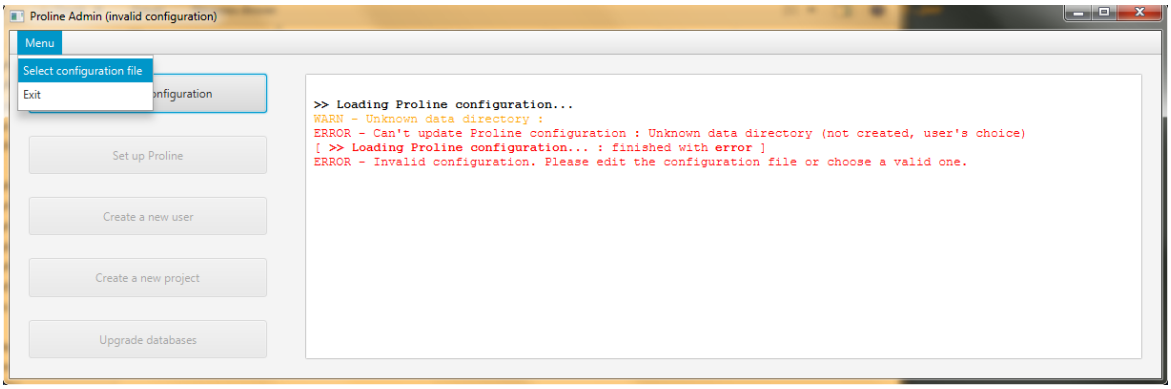
ProlineAdmin GUI usage

The default configuration file `config/application.conf` is loaded. You can alternatively edit this file (see Configuring ProlineAdmin section below) or select another `.conf` file of the same format.

To edit default file, press the “Edit Proline configuration” button. You can now edit your file in the newly opened window, and save it.

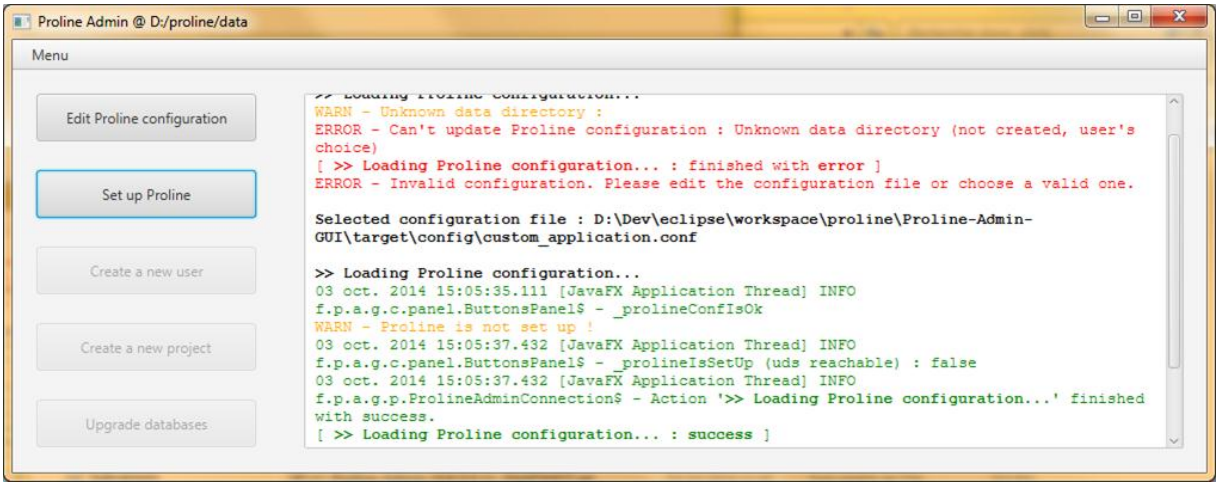


To load a `.conf` file, use the menu on the top left and select your file in the file browser.


Proline Admin (invalid configuration)
Menu
Select configuration file
Exit
Set up Proline
Create a new user
Create a new project
Upgrade databases
>> Loading Proline configuration...
WARN - Unknown data directory :
ERROR - Can't update Proline configuration : Unknown data directory (not created, user's choice)
[>> Loading Proline configuration... : finished with error]
ERROR - Invalid configuration. Please edit the configuration file or choose a valid one.

Finish the datastore setup by clicking the newly available button “Set up Proline”.

Note: This functionnality will be disabled if Proline is already set up or if Proline configuration is invalid.


Proline Admin @ D:/proline/data
Menu
Edit Proline configuration
Set up Proline
Create a new user
Create a new project
Upgrade databases
WARN - Unknown data directory :
ERROR - Can't update Proline configuration : Unknown data directory (not created, user's choice)
[>> Loading Proline configuration... : finished with error]
ERROR - Invalid configuration. Please edit the configuration file or choose a valid one.
Selected configuration file : D:\Dev\eclipse\workspace\proline\Proline-Admin-GUI\target\config\custom_application.conf
>> Loading Proline configuration...
03 oct. 2014 15:05:35.111 [JavaFX Application Thread] INFO f.p.a.g.c.panel.ButtonsPanel$ - _prolineConfIsOk
WARN - Proline is not set up !
03 oct. 2014 15:05:37.432 [JavaFX Application Thread] INFO f.p.a.g.c.panel.ButtonsPanel$ - _prolineIsSetUp (uds reachable) : false
03 oct. 2014 15:05:37.432 [JavaFX Application Thread] INFO f.p.a.g.p.ProlineAdminConnection$ - Action '>> Loading Proline configuration...' finished with success.
[>> Loading Proline configuration... : success]

Using the command line interface (ProlineAdmin)

Edit the configuration file `config/application.conf` located in the ProlineAdmin folder (see Configuring ProlineAdmin section below). Then perform the datastore setup by running the dedicated script.

Windows users

The ProlineAdmin program files are located in the “.\admin” sub-folder of the Proline installation directory. You should find the following script in this folder:

```
> setup_proline.bat
```

Linux users or manual installation

Execute the `setup_proline.sh` script located in the folder obtained after Proline Admin GUI archive file extraction.

Configuring ProlineAdmin

Modify the following lines to fit your DBMS configuration:

```
proline-config {
 driver-type = "postgresql" // valid values are: h2, postgresql or sqlite
 data-directory = "/Path/to/Proline/Data" //Not used actually...
}

auth-config {
 user="proline_user" // !! SET TO Database Proline user login
 password="proline_user_password" // !! SET TO Database Proline user
password
}

host-config {
 host="your_postgresql_server_host" //!! Do NOT put "localhost", but the
real IP address or fully qualified name
 port="5432" //or other port used to access your DBMS
}
```

Note: default naming scheme of databases created by Proline can be modified by editing `config/application-<dbtype>.conf` file.

Configuring the Proline Server

Locating the server folder

Windows users

The server program files are located in the ".\ws" sub-folder of the Proline installation directory.

Linux users or manual installation

Open the folder where you have unzipped the "Proline Server" archive. The Proline server folder should contain a sub-folder named `ProlineWeb-Core-<x.y.z>`.

Editing the configuration file

The configuration file is located at `<proline_server_folder>\ProlineWeb-Core-<x.y.z>\Proline\WEB-INF\classes`.

Configuring the datastore

Edit the `application.conf` file in the same way you did it for ProlineAdmin (see Setting up the Datastore). If your configuration is valid, the Proline Server will be able to use the datastore you've created using Proline Admin.

Configuring the mount-points

Result identification files (Mascot dat or OMSSA) as well as mzDB files (for the XIC Quantitation process) are only browsed from Proline Server side.

Administrator must configure the target directory/ies in the entry `mount_points` in the `application.conf` file

Mascot dat or OMSSA path should be configured in `result_files` sub-entry, administrator can add one or more mappings as `label = "<absolute/directory/path>"`. mzDB files path should be set under `mzdb_files` sub-entry.

Label can be any valid string chosen by Administrator to help user identify mount_point. If multiple repositories are defined, labels must be different.

Configuration examples:

```
mount_points {

 result_files {
 mascot_data = "Z:/" //under window environement
 omssa_data = "/local/omssa/data" //under linux environement
 ...
 }
 ...

 mzdb_files {
 }

}
```

Running the server

Administrator can change default amount of memory used by the server in the `jetty-runner.bat` /`jetty-runner.sh` file. If the server is configured with large amount of memory, it is recommended to increase this value. Change the value of -Xmx option: Xmx4g ⇒ -Xmx9g to pass from default 4 GO to 8GO.

Run `jetty-runner.bat` (or jetty-runner.sh on linux system) to start the jetty server. You should now be able to access ProlineWeb-Core by typing http://localhost:8080/proline or http://<host>:8080/proline in your favourite browser. The following message must appear:

```
ProlineWeb-Core working !

Number of IVersion services : <X>
fr.proline.core.wsl.Version Module: ProlineWeb-Core Version: <XXX>
fr.proline.module.parser.omssa.Version Module: PM-OmssaParser Version:
<YYY>
fr.proline.module.parser.mascot.Version Module: PM-MascotParser
Version:<XYZ>
fr.proline.admin.Version Module: Proline-Admin Version: <ZYW>
fr.proline.util.ScalaVersion Module: ProFI-Commons-Scala Version: <YZX>
fr.proline.util.JavaVersion Module: ProFI-Commons-Java Version: <YXZ>
fr.proline.core.service.Version Module: Proline-OMP Version: <WYZ>
```

Installing and configuring the Sequence Repository

Even if this is an optional module it is recommended to install it, mostly if you want to view the proteins sequences in the user interfaces!

It can be installed on the same machine running the Proline Server. However as this module will parse the mascot fasta files to extract sequence and description from it, it will be more efficient if installed on the computer executing your Mascot Server. In any case, you should also be able to access to the PostgreSQL server from the computer where Sequence Repository is installed.

Sequence Repository installation

Windows users

Select this component from the wizard of the automated installer. The corresponding program files will be located in the ".\seqrepo" sub-folder of the Proline installation directory.

Linux users / manual installation

This module is distributed as an archive file (embedded in Proline Server archive) and need to be extracted in your preferred folder to be installed.

Configuration

Configuration files are located under the "<seqrepo_folder>/config".

Datastore description

pg_uds.properties file define datastore description to access to the UDS database (for postgresql database):

```
javax.persistence.jdbc.driver=org.postgresql.Driver
javax.persistence.jdbc.url=jdbc:postgresql://<host>:<port>/<uds_db>
javax.persistence.jdbc.user=<user_proline>
javax.persistence.jdbc.password=<proline_user_password>
```

Note :

- If you didn't change the default naming scheme of databases the <uds_db>= 'uds_db' so

  ```
  javax.persistence.jdbc.url=jdbc:postgresql://<host>:5432/uds_db
  ```

- proline_user_password and user_proline are the same as specified in `application.conf` for Proline Admin or Proline WebCore

Protein description parsing rule

As this module is used to extract Protein Sequence, description from a fasta file for a specific protein accession, it is necessary to configure the rule used to parse the protein ACC, from fasta description line. This is similar to the rules specified in Mascot Server. To do this, *retrieve-service.properties* file should be edited. In this file it is necessary to escape (this means prefix with '\') some characters: '\', ':' and '='

```
# Name of the UDS Db configuration file (Java properties format)
fr.proline.module.seq.udsDbConfigurationFile=pg_uds.properties

# Paths must exist (regular file or directory) and multiple paths must be
separated by ';' character
fr.proline.module.seq.localFASTAPaths=Y\:\\sequence;D\:\\Temp\\Admin\\FAS
TAs

# Java Regex with capturing group for SEDbInstance release version string
(CASE_INSENSITIVE)
fr.proline.module.seq.defaultReleaseRegex=_(?:D|(?:Decoy))_(.*)\\.fasta

# UniProt style SEDb (FASTA file name must contain this Java Regex
CASE_INSENSITIVE) multiple Regex separated by ';' character
fr.proline.module.seq.uniProtSEDbNames=\\AUP_;ISA_D

# Java Regex with capturing group for SEDbIdentifier value (without
double quote)
# UniProt EntryName ">\\w{2}\\|[^\\|]*\\|(\\S+)"
# UniProt UniqueIdentifier (primary accession number)
">\\w{2}\\|([^\\|]+)\\|"
# GENERIC Regex ">(\\S+)"
fr.proline.module.seq.uniProtSEDbIdentifierRegex=>\\w{2}\\|[^\\|]*\\|(\\S
+)
```

Note:

- fr.proline.module.seq.localFASTAPaths : only one instance should be defined. For linux system, fr.proline.module.seq.localFASTAPaths=/local/data/fasta;/local/mascot/sequence
- fr.proline.module.seq.defaultReleaseRegex : Regular expression to extract release version (CASE_INSENSITIVE) from the fasta files.
- fr.proline.module.seq.uniProtSEDbNames : Regular expression to identify Uniprot like fasta. The entry of these files will be parse using specific rule (fr.proline.module.seq.uniProtSEDbIdentifierRegex) to extract protein accession.

For other fasta file the protein accession will be extract by using string before first blank.

Installing Proline Studio

- Proline Studio application distribution is a zip file that must be extracted on each client computer.

Installing and configuring Proline Web Desktop

- Install, Configure and launch the Desktop

Installating Proline Web

- The Proline Web eXtension (PWX) is based on the use of MongoDB database engine. You need to download it and install it either on the computer which will host the PWX server, or any other network-accessible computer. You will find the installation files on this page : http://www.mongodb.org/downloads
- Download and unzip the PWX archive.

NOT AVAILABLE YET: The automated installer embeds several components of the Proline Suite. You may only install the Proline Web component, but if you want you can perform a full installation. It will install Proline-Admin and Proline-WebCore in the bin directory of Proline Web.

Configure the Server

If you installed your MongoDB database on a different computer than the PWX server, you'll need to edit the PWX configuration file:

- Go to the installation directory of your PWX server
- Go to the “conf” folder and open the “application.conf” file with any text editor (like Window default Notepad)
- Edit the mongodb.servers and cache.mongodb.servers parameters by setting the host name and the port number corresponding to your MongoDB server (default MongoDB port is 27017)

If MongoDB is installed on the same computer as the PWX server, you don't need to configure anything at this time.

Launch The Server

- First, please make sure that MongoDB is running. If it's not, please start it manually.
- In order to start the PWX server, go to its installation folder. On Windows platforms launch the “start.bat” script. On Linux platforms execute the “start.sh” script (TODO: create this script).

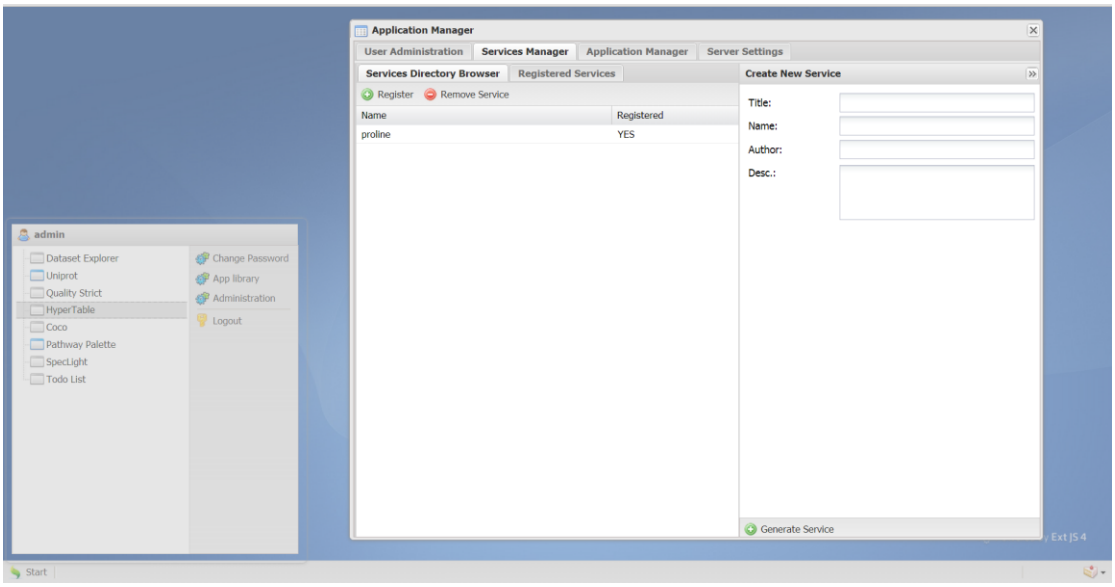
Connect to the Proline Desktop

Once PWX is running, you can connect to the Proline Web Desktop by opening a Web Browser and go to the address of this form: “name-of-the-machine:9000” or “local.ip.of.the.machine:39000” (for instance 192.168.0.30). The default user is “admin”

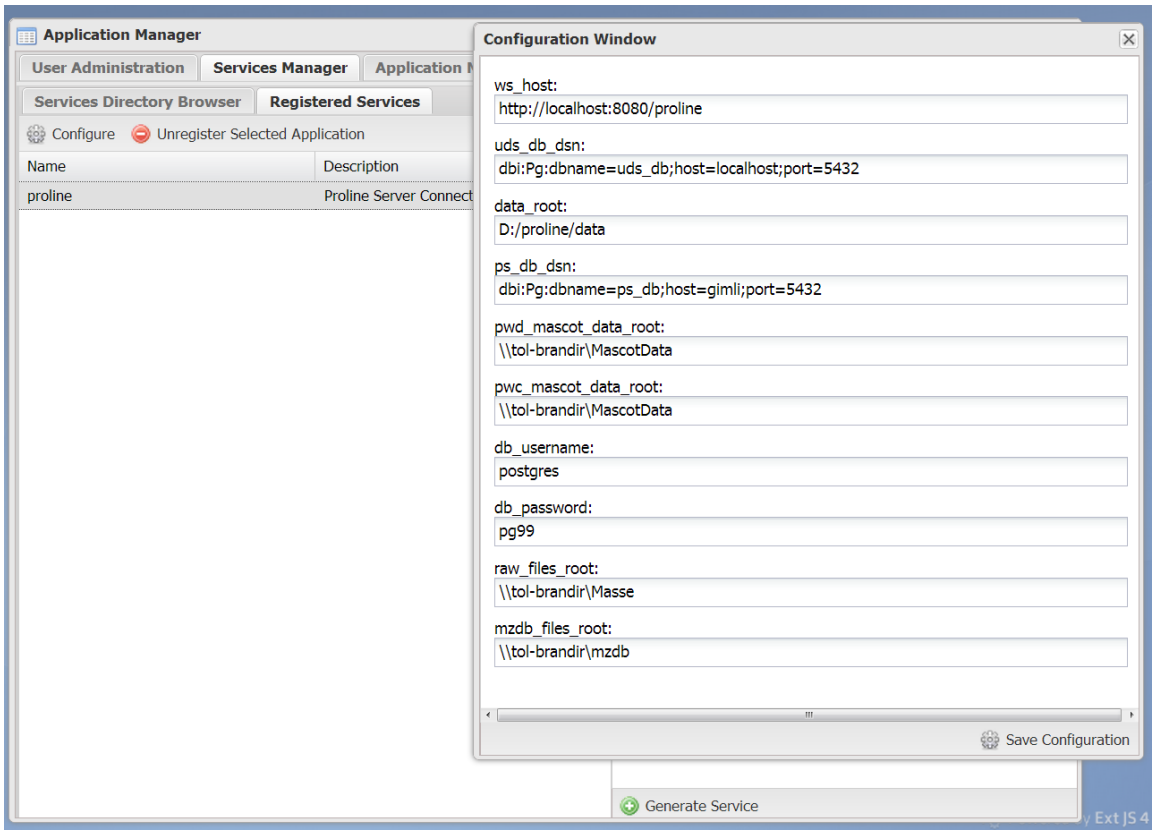
and its password is “proline”. Don't forget to change its password from the start menu button once you're logged in.

Setup the Proline Service

- The connection between the desktop and the proline core server is provided by the “proline” service. This service is included with the Proline Web eXtension server, but it needs to be configured. The following steps will explain how :
- To set it up, go to “Start”>“Administration”>“Services Manager”. On the “Services Directory Browser”, you should see the “proline” service appear in the the table.



- Select “proline” in the Grid Table, and then click on “Configure”



- Here are some param values you can use :
  - ws_host is the adress of the Proline Web Core server. Use the name or the IP adress of the server to set the URL. Examples :
    - http://servername:8080/proline
    - http://localhost:8080/proline
    - http://198.0.13.37/proline (replace 198.0.13.37 by the right IP adress)
  - uds_db_dsn is the UDS database connexion configuration (DSN stands for Data Source Name). It has the following format **dbi:** + name of the Driver (**Pg** for PostgreSQL, **SQLite** for SQLite) + **:db_name=** + name of the Postgre SQL database (**uds_db** or path to the SQLite file **D:/path/to/uds_db.sqlite**) + **;host=** + Postgre SQL erver adress or name + **;port=** + port of the Postgre SQL server. The host and port params are not needed if you use a SQLite database. Here are some config examples :
    - dbi:Pg:dbname=uds_db;host=localhost;port=5432
    - dbi:SQLite:dbname=D:/proline/data/uds_db.sqlite
  - data_root is the directory where the user projects data will be stored. You can use something like **D:/proline/data**. Make sure this directory exists on your disk, Proline won't create it automatically if it doesn't.
  - ps_db_dsn is equivalent to the uds_db_dsn param but points to the PS Database. Set up the connection config the same way you did it for the UDS Database.
  - pwd_mascot_data_root is the Directory where the PWX server will browse mascot result files. Set it up with a simple path like **D:/proline/mascot/data** or a network adresse like **\\servername\mascot\data**. Make sure this directory exists on your disk, Proline won't create it automatically if it doesn't.

    - pwc_mascot_data_root is the path from which the Proline Web Core will access this directory. You should leave it blank if you configured it in the Proline Web Core config file (result_files > root_folder param). If you didn't specify any path there, then you must enter one here in the Proline Service config. This param can also be useful if you installed PWX and Proline Web Core on two different servers, and if one of them runs on Linux, for example. These two paths (pwd_mascot_data and pwc_mascot_data must point to the same folder. There are two of them because you need the PWX server and the Proline Web Core server may not access it via the same way. Fill it the right way so the both servers can access the same directory. If they are on the same network and use the same OS (i.e Windows), you can set up the same path on both fields.
    - db_username is the user name used by the PostgreSQL connection. not needed if you use SQLite. The default PostgreSQL user is " *postgres* ".
    - db_password is the password you gave to the PostgreSQL user specified above.
    - raw_files_root is the path to your .raw files root directory
    - mzdb_files_root is the path to your .mzdb files root directory

- You must set up the right connection information and data directories in order to access the Proline Core server properly
- You must set up two path to the mascot data root folder : one for the desktop (pwd_mascot_data_root) and one for jetty (pwc_mascot_data_root).
- Set up the SQL Server connection settings, and the path to the raw files and mzdb files root directory.
- Once it's done, simply click "Save Configuration"
- To make sure that the "admin" user is registered in the UDS DB, you need to logout and login, in order to make the Proline Service to check your status in the UDS DB, and create your account if you're not registered yet

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How to

Note: Read the Concepts & Principles documentation to understand main concepts and algorithms used in Proline.

Proline Admin

- Create a Proline User
- Create a Proline Project

Proline Studio

Creation/Deletion

- Open a session and access to my projects
- Create a new project

- Create a Dataset
- Import a Search Result
- Delete Data
- Connection Management

Display

- Display Peptides/PSM or Proteins of a Search Result
- Display PSM, Peptides or Protein Sets of an Identification Summary
- Display Search Result & Identification Summary additional information
- Display Spectral Counts
- Display XIC
- Create and Save a User Window
- List of Abbreviations
- Frame Toolbars Functionalities
- Filter tables
- Search tables
- Graphics : Scatter Plot / Histogram
- Statistical Reports (MSDiag)

Save, import and export

- Import Mascot/OMSSA result file
- Export data

Algorithm and other operation

- Validate a Search Result
- Change Typical Protein of a Protein Set
- Merge
- Data Mixer

Quantitation

- Spectral Count
- XIC
- Refine Protein Sets Abundances

Proline Web

Workflow

- Open a session and access to my projects
- Register and pair Raw & MzDB files
- Create a new project
- Import Result Files
- Create an Identification Dataset
- Validate a Search Result
- Create a Quantitation

- Delete Datasets

Users management

- Create a User

Display

- Display peptides and/or PSM in identification result
- Display proteins sets in Identification Summary
- Display Identification Summary additional information

Save, import and export

- Export data
- Import Result Files

Algorithm and other operation

- Validate a Search Result

Create a Proline project

Command line (ProlineAdmin)

Run the following command line from the ProlineAdmin directory:

- Windows :

```
run_cmd.bat create_project -oid <owner_id> -n <project_name> -desc
<project_description>
```

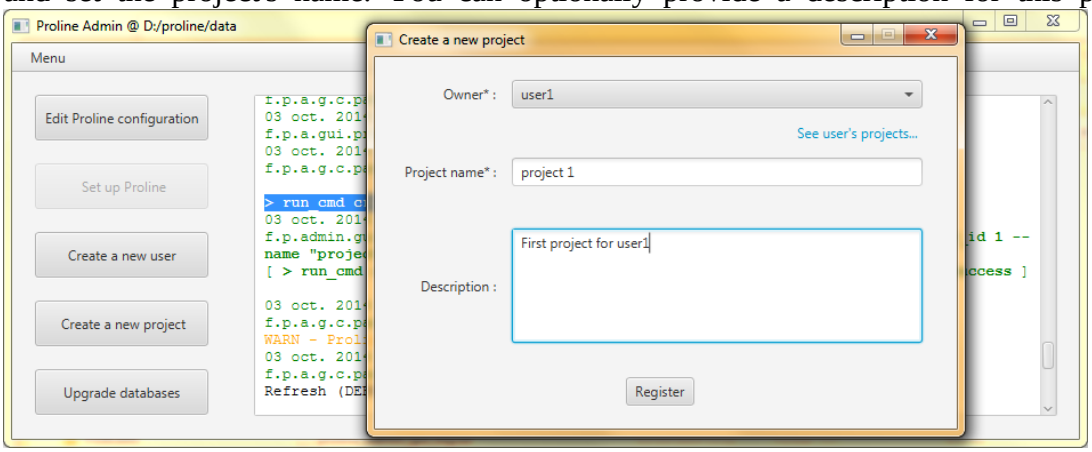
- Linux :

```
sh run_cmd.sh create_project -oid <owner_id> -n <project_name> -desc
<project_description>
```

Note: The project's description is optional.

From graphical tool ProlineAdmin GUI

Click on the “Create a new project” button, then select the project's owner from users list and set the project's name. You can optionally provide a description for this project.



Note: Since the project's owner must be provided, this functionnality will be disable if Proline is not set up or if no Proline user is registered yet (see how to set up Proline and create a Proline user).

Create a Proline user

Command line (ProlineAdmin)

You can create a proline user with the Proline Admin “RunCommand” script. Open a command line window (Maj + Right Click on Windows) and type the following command:

- Windows :

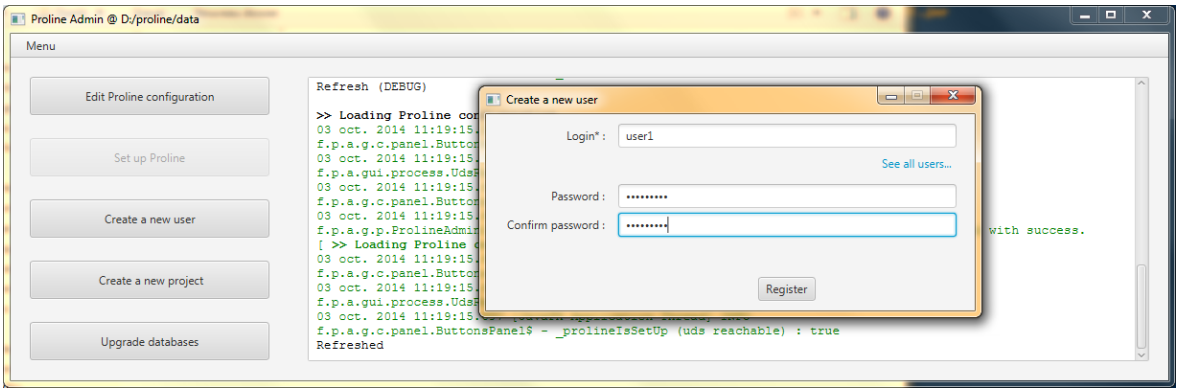
```
run_cmd.bat create_user -l <user_login> -p <user_password>
```

- Linux :

```
sh run_cmd.sh create_user -l <user_login> -p <user_password>
```

From graphical tool ProlineAdmin GUI

You can also use the ProlineAdmin graphical interface: open ProlineAdmin GUI and click on the “Create user” button. A new window allows you to set the new user's name and password (with password verification).



Note: This functionality will be disable if Proline is not set up (see how to set up Proline).

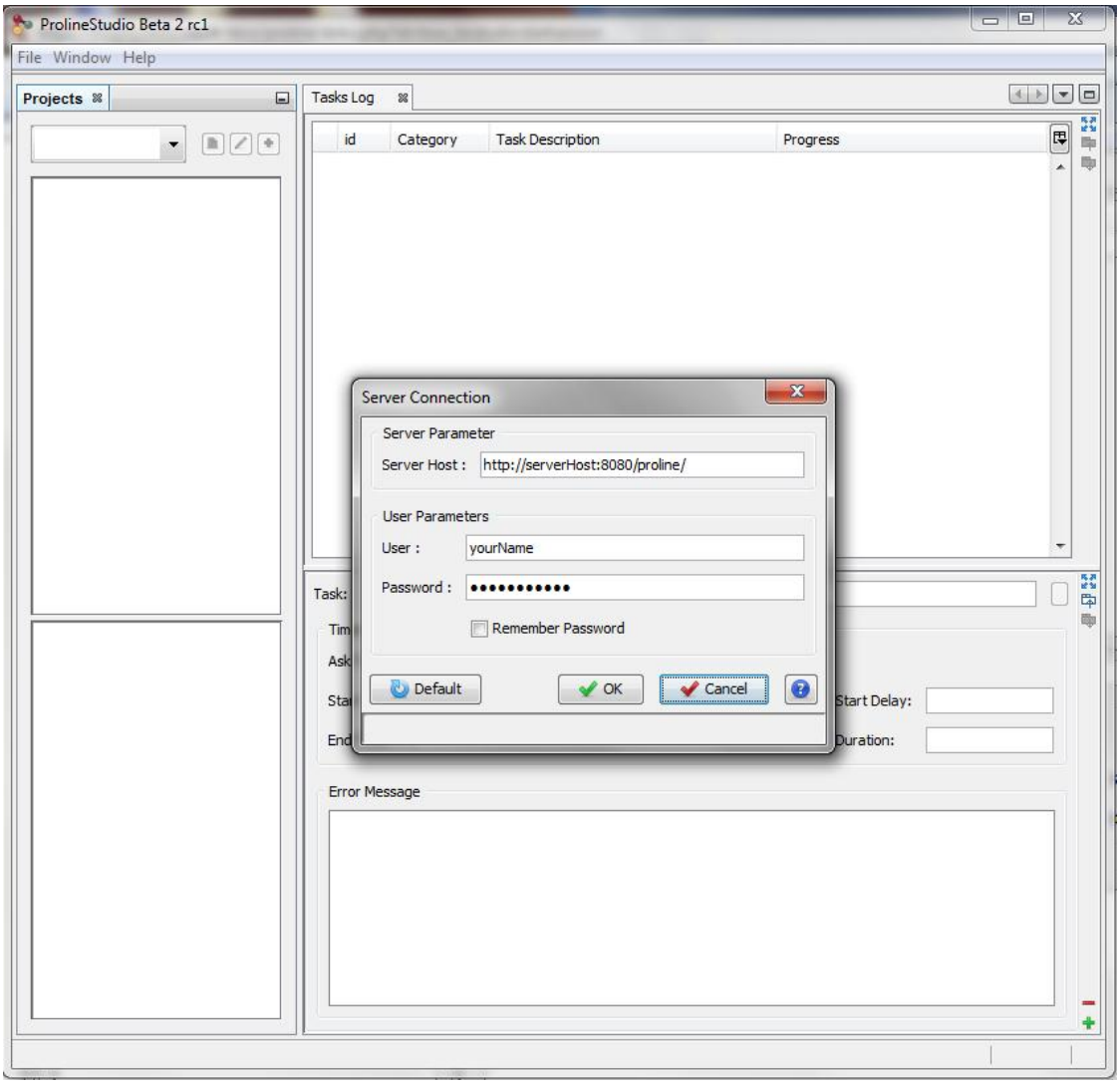
From Proline Web Desktop

You can also create users from the Proline Web Desktop administration interface: Create a User and synchronise it with the Proline Core UDS Database

Proline STUDIO

Server Connection

When you start Proline Studio for the first time, the Server Connection Dialog is automatically displayed.



You must fill the following fields:

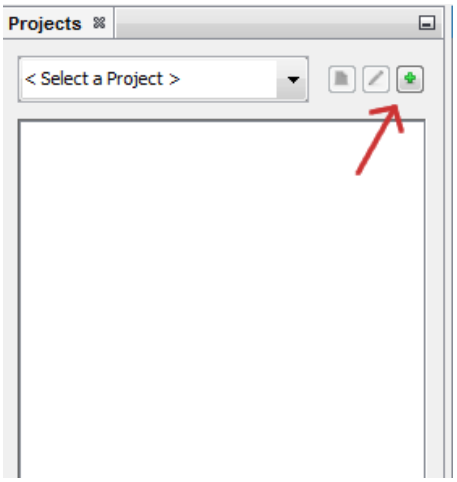
- **Server Host**: this information must asked to your IT Administrator.

- **Project User**: your name (an account must have been previously created by the IT Administrator).

- **Password**: password corresponding to your account.

If you check “Remember Password”, the password will be saved. So, when you will re-start the application, Proline Studio will automatically connect to the server and load your projects without opening the Server Connection Dialog.

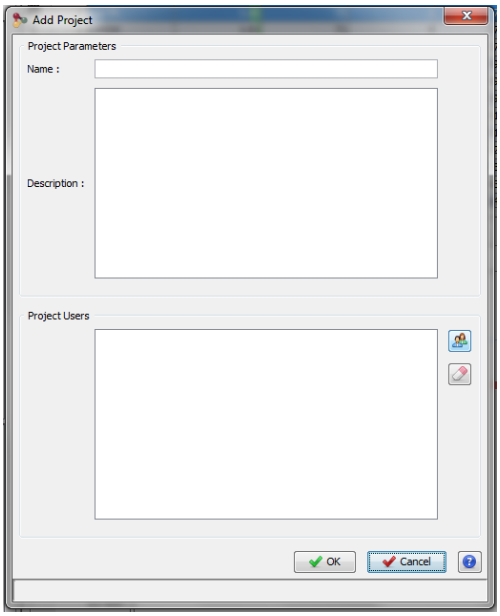
Create a New Project



To create a Project:

- Click on “+“button at the right of the Project Combobox.

The Add Project Dialog is opened.



Fill the following fields:

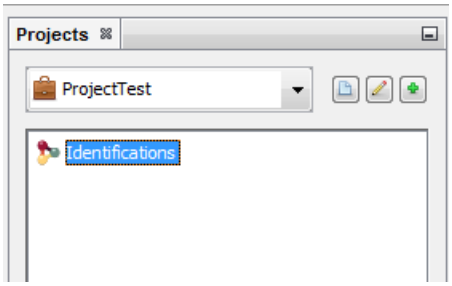
- **Name**: name of your project

- **Description**: description of your project

You can specify other people to share this new project with them.

Then click on OK Button

Creation of a Project can take a few seconds. During its creation, the Project is displayed grayed with a small glasshour over it.



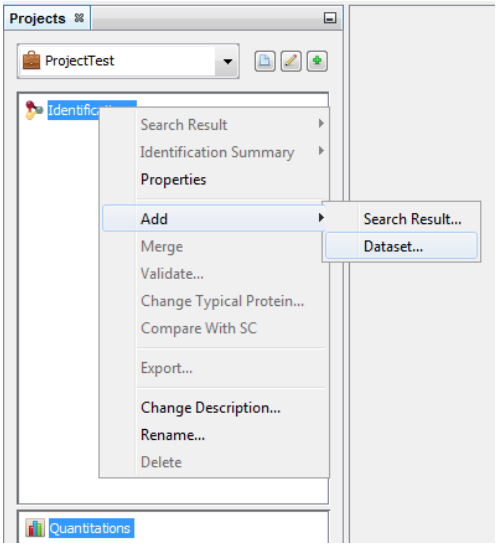
Create a Dataset

You can create a Dataset to group your data.

To create a Dataset:

- right click on Identifications or on a Dataset to display the popup.

- click on the menu “Add > Dataset…”

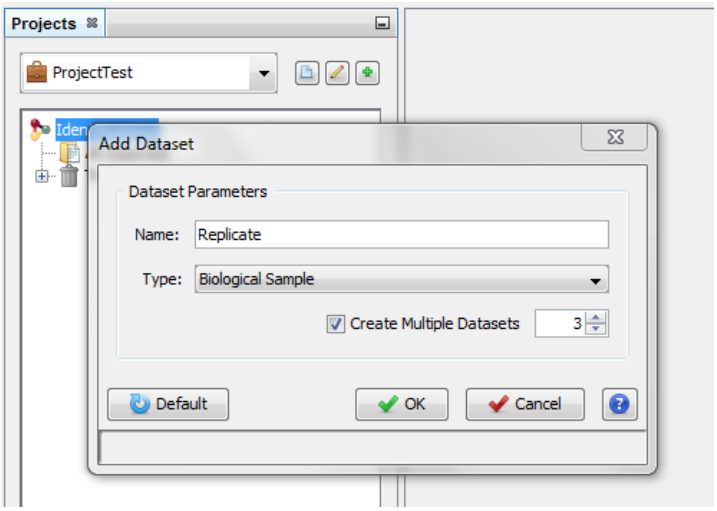


On the dialog opened:

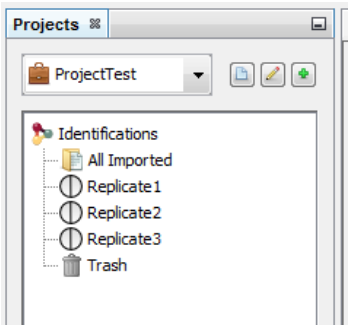
- fill the name of the Dataset

- choose the type of the Dataset

- optional: click on “Create Multiple Datasets” and select the number of datasets you want to create



Let's see the result of the creation of 3 datasets named “Replicate”:



Import a Search Result

There are two possibilities to import Search Results:

- import multiple Search Results in “All Imported” and put them later in different datasets.

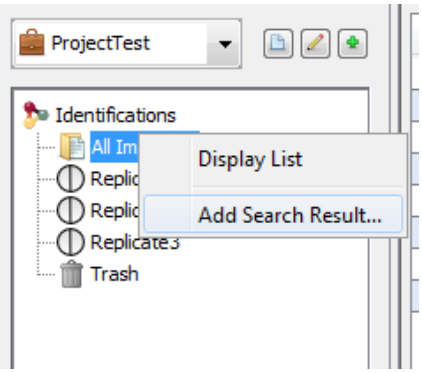
- import directly a Search Result in a dataset.

Import in "All Imported"

To import in “All Imported”:

- right click on “All Imported” to show the popup

- click on the menu “Add Search Result…”



In the Import Search Results Dialog:

- select the file(s) you want to import thanks to the file button (the Parser will be automatically selected according to the type of file selected)

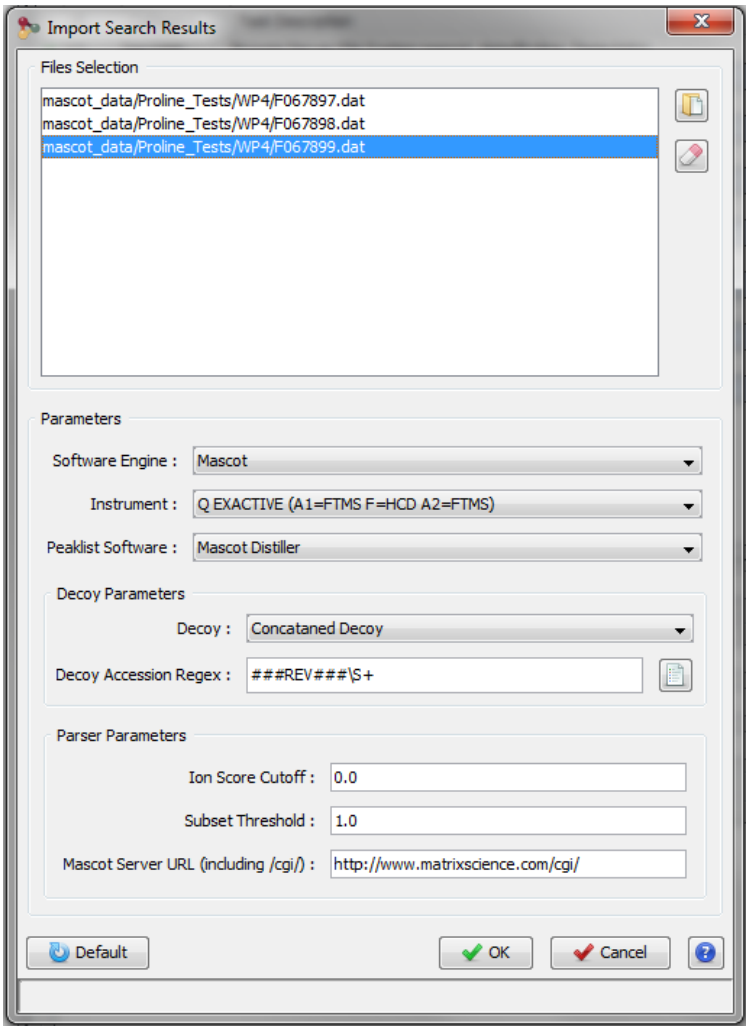
- select the different parameters

- click on OK button

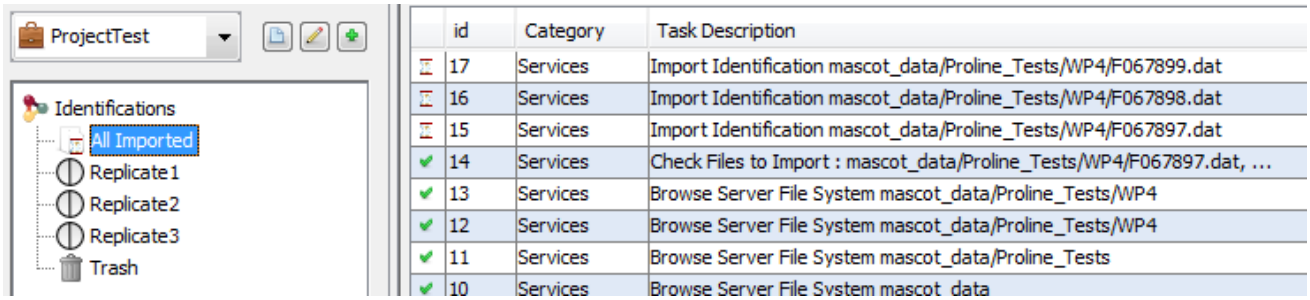
Note 1: You can only browse the files accessible from the server according to the configuration done by your IT Administrator. Ask him if your files are not reachable. (Look for *Setting up Mount-points* paragraph in Installation & Setup page)

Note 2: The **“Save Spectrum Matches”** option does no longer exist. The Spectrum matches can be generated on demand when the Search Result is imported.

Note 3: Proline is able to import OMSSA files compressed with BZip2.

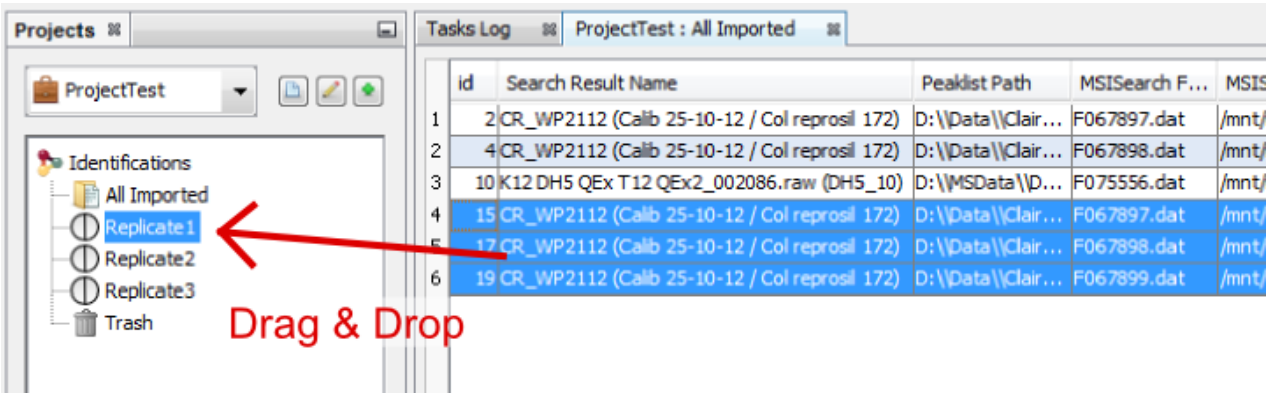


Importing a Search Result can take some time. While the import is not finished, the "All Imported" is shown grayed with an hour glass and you can follow the imports in the Tasks Log Window (Menu Window > Tasks Log to show it).



To show all the Search Results imported, double click on "All Imported", or right click to popup the contextual menu and select "Display List"

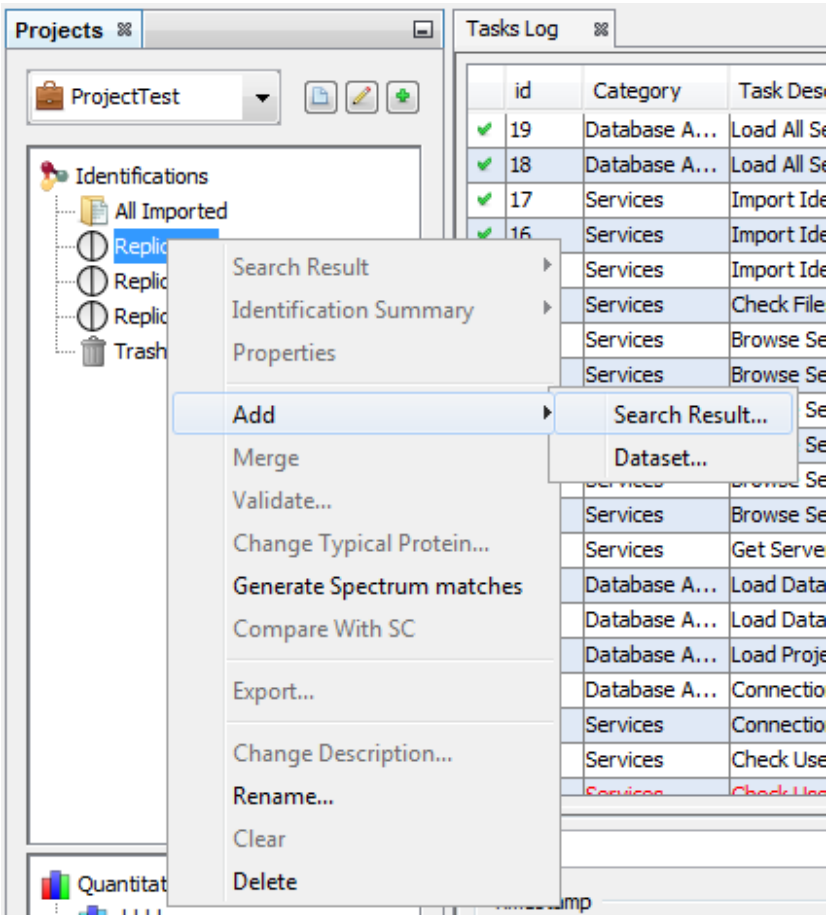
From the All Imported window, you can drag and drop one or multiple Search Result to an existing dataset.


Projects
Tasks Log
ProjectTest : All Imported
ProjectTest
Identifications
All Imported
Replicate1
Replicate2
Replicate3
Trash
Drag & Drop
id
Search Result Name
Peaklist Path
MSISearch F...
1 2 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\Data\Clair... F067897.dat
2 4 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\Data\Clair... F067898.dat
3 10 K12 DH5 QEx T12 QEx2_002086.raw (DH5_10) D:\MSData\D... F075556.dat
4 15 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\Data\Clair... F067897.dat
5 17 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\Data\Clair... F067898.dat
6 19 CR_WP2112 (Calib 25-10-12 / Col reprosil 172) D:\Data\Clair... F067899.dat

Import directly in a Dataset

It is possible to import a Search Result directly in an Dataset. In this case, the Search Result is avaible in "All Imported" too.

To import a Search Result in a Dataset, right click on a dataset and then click on "Add > Search Result…" menu.



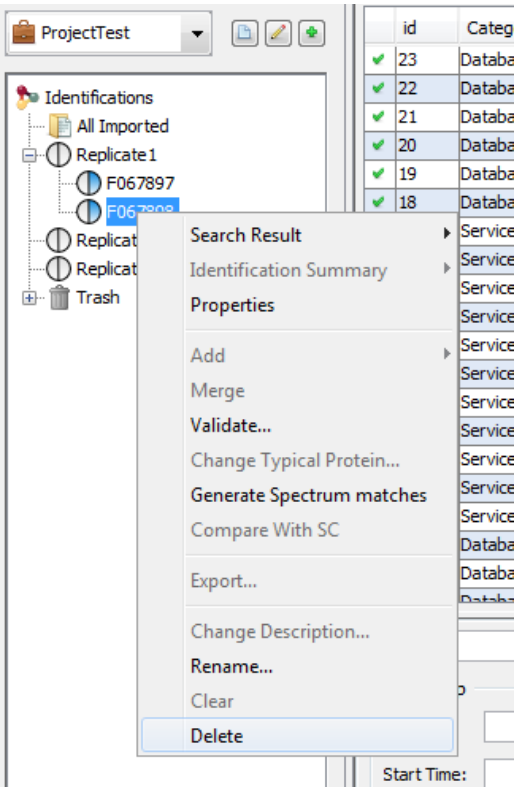
Delete Data

You can delete Search Results, Identification Summaries and Datasets in the data tree. You can also delete XIC or Spectral Counts in the quantitation tree.

There are two ways to delete data: use the contextual popup or drag and drop data to the Trash.

Delete Data from the contextual popup

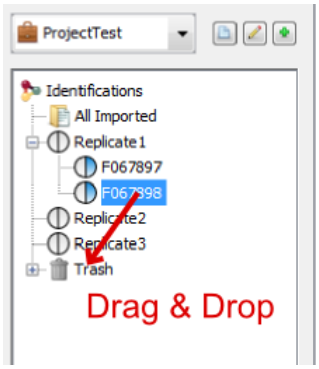
Select the data you want to delete, click the mouse right button to open the contextual menu and click on delete menu.



The selected data is put in the Trash. So it is possible to restore it while the Trash has not been emptied.

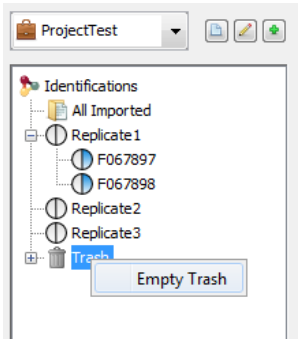
Delete Data by Drag and Drop

Select the data you want to delete and drag it to the Trash. It is possible to restore data while the Trash has not been emptied



Empty the Trash

To empty the Trash, you have to Right click on it and select the “Empty Trash” menu.



(In fact, for the moment, Search Results are not completely removed, you can retrieve them from the “All Imported” window)

Delete a Project

It is not possible to delete a Project by yourself. If you need to do it, ask to your IT Administrator

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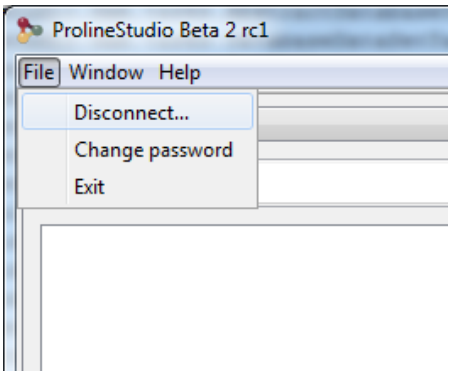
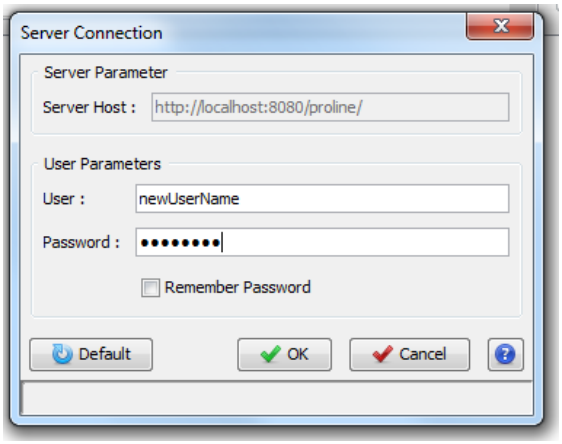
Connection Managment

Once user is connected (see Server Connection), it is possible to:

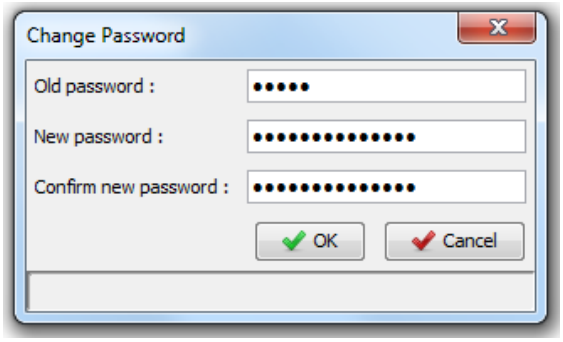
- Disconnect



- Reconnect with a different login



- Change password



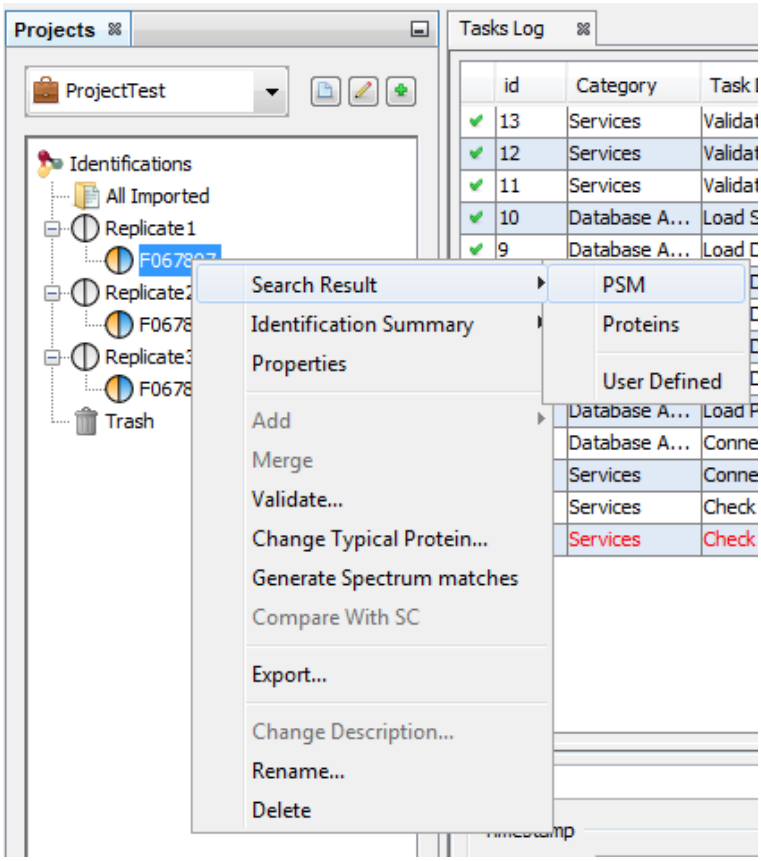
Display peptides/PSM or Proteins of a Search Result

Functionality Access

To display data of a Search Result:

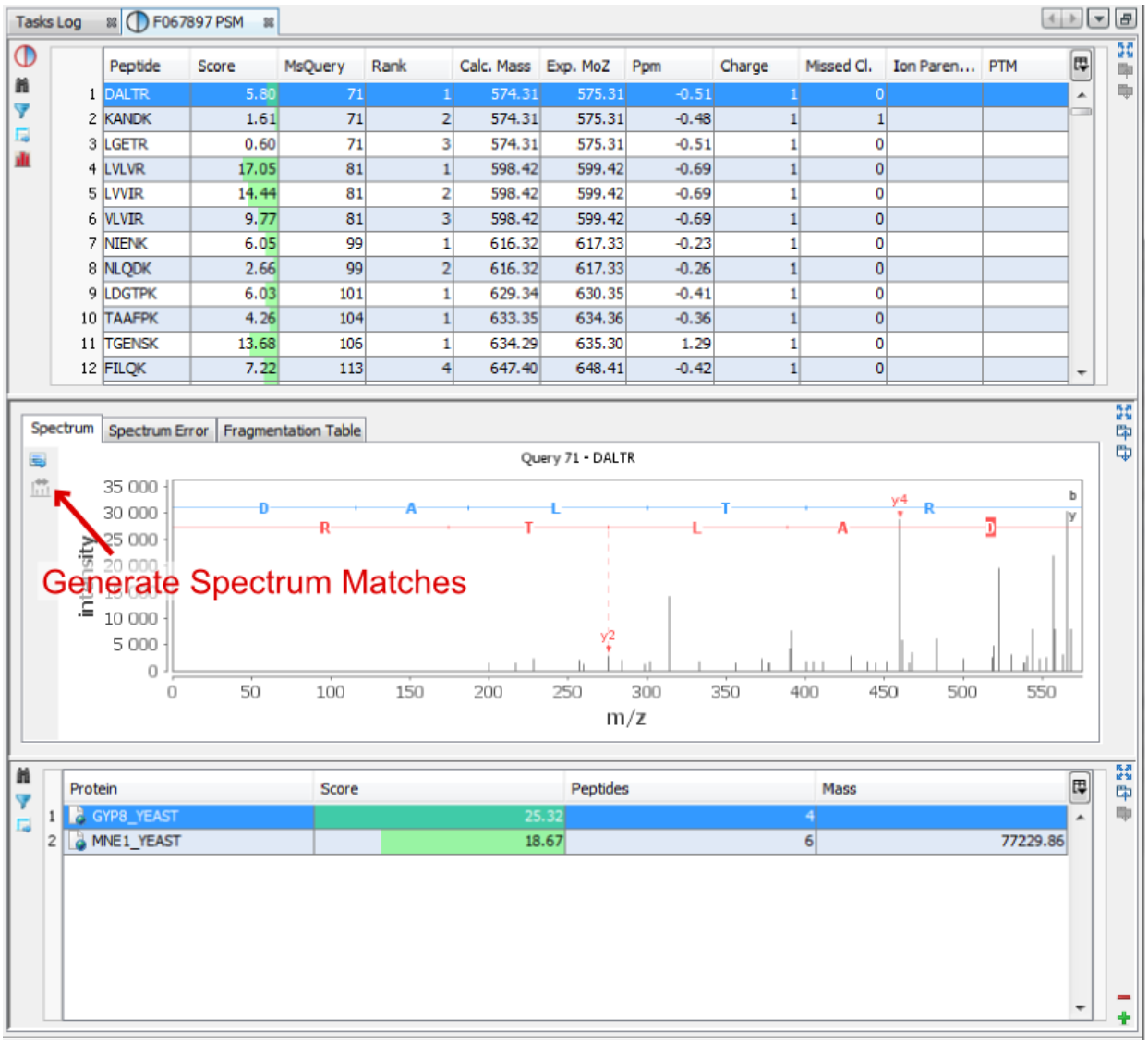
- right click on a Search Result

- click on the menu “Search Result >” and on the sub-menu “PSM” or “Proteins”



Peptides/PSM Window

If you click on PSM sub-menu, you obtain this window:



Upper View: list of all PSM/Peptides.

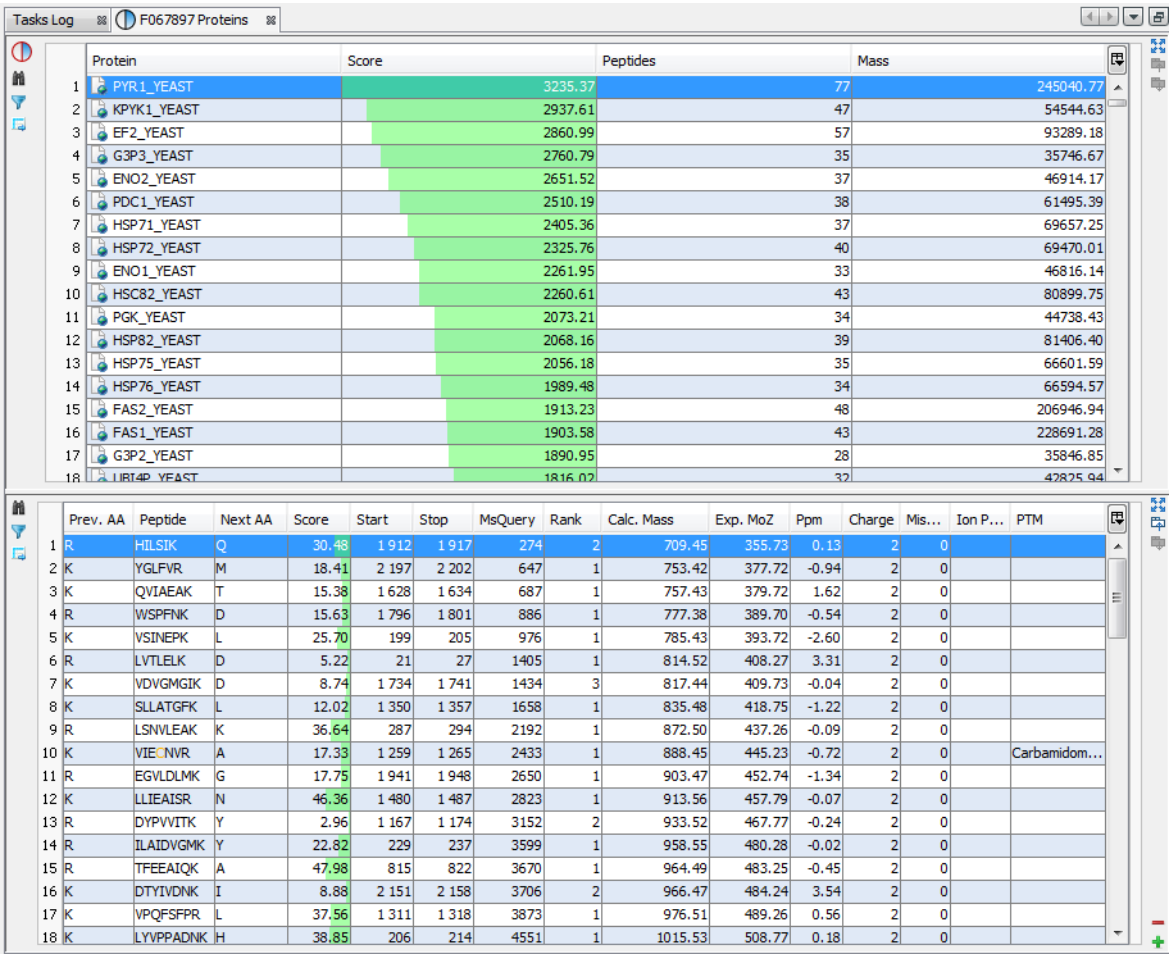
Middle View: Spectrum, Spectrum Error and Fragmentation Table of the selected PSM. If no annotation is displayed, you can generate Spectrum Matches by clicking on the according button

Bottom Window: list of all Proteins containing the currently selected Peptide.

Note: Abbreviations used are listed here

Proteins Window

If you click on Proteins sub-menu, you obtain this window:



Upper View: list of all Proteins

Bottom View: list of all Peptides of the selected Protein.

Note: Abbreviations used are listed here

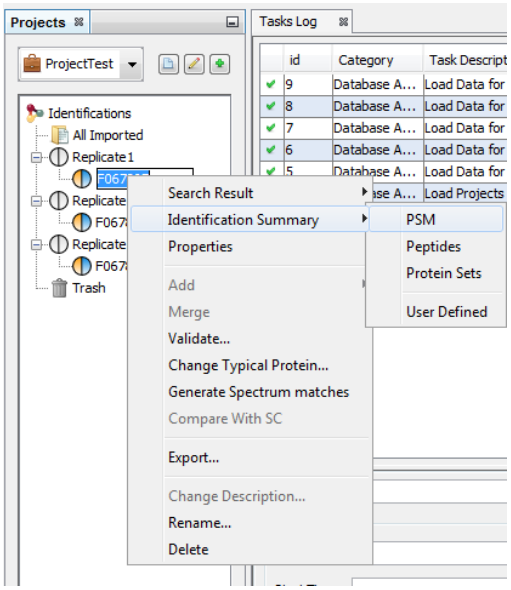
Display PSM, Peptides or Protein Sets of an Identification Summary

Functionality Access

To display data of an Identification Summary:

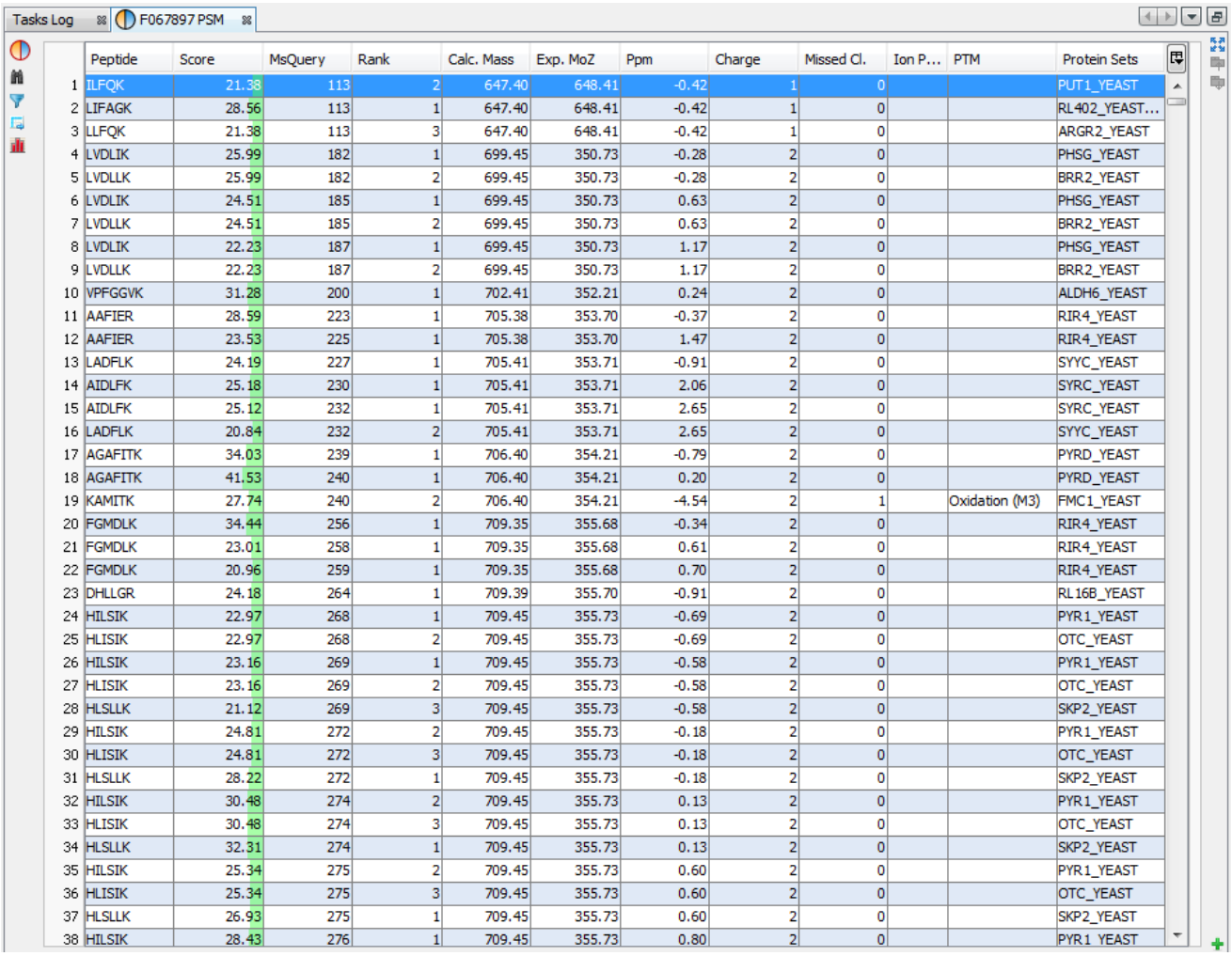
- right click on an Identification Summary

- click on the menu “Identification Summary >” and on the sub-menu “PSM”, “Peptides” or “Protein Sets”



PSM Window

If you click on PSM sub-menu, you obtain this window:



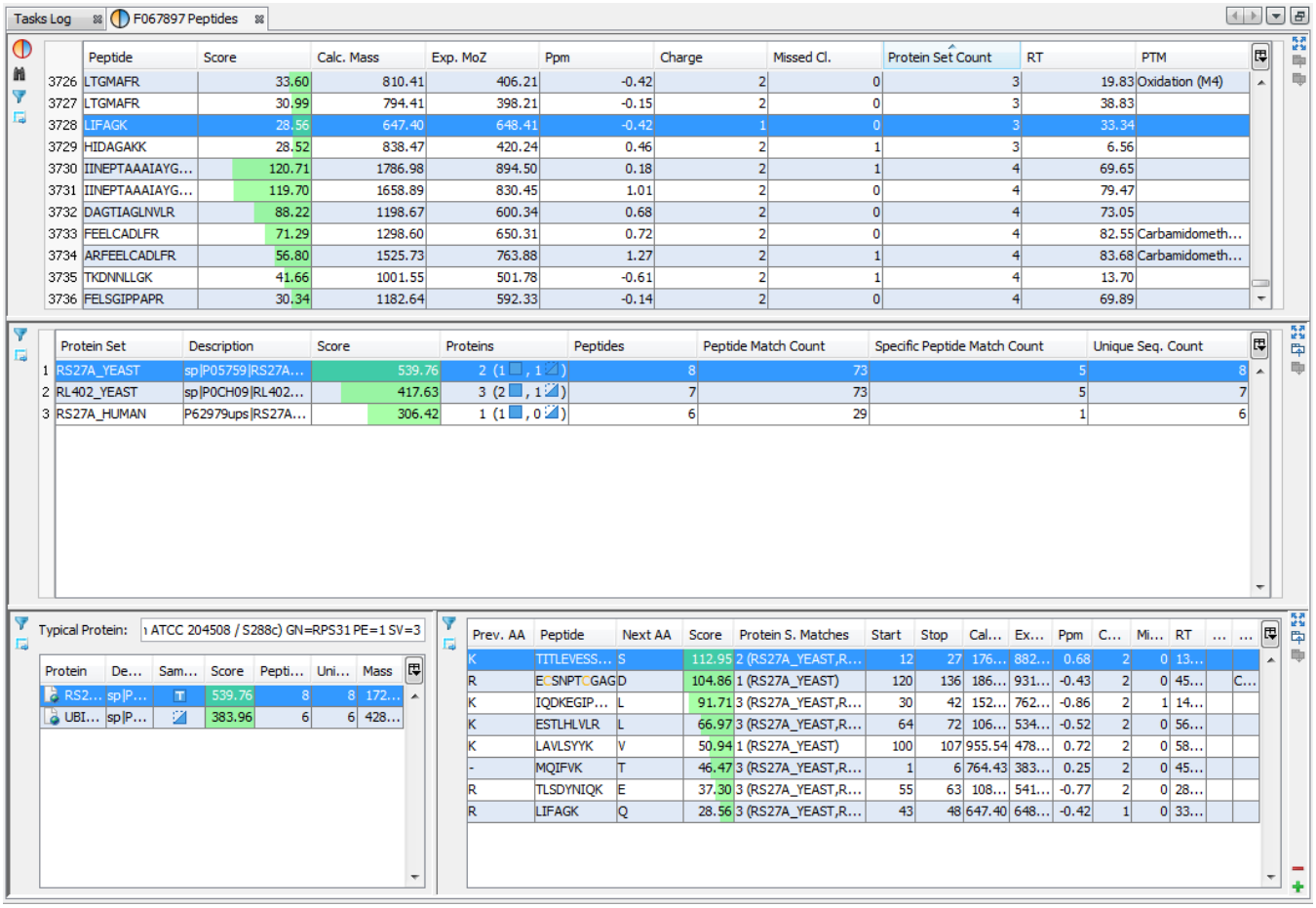
Tasks Log | F067897 PSM

	Peptide	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion P...	PTM	Protein Sets
1	ILFQK	21.38	113	2	647.40	648.41	-0.42	1	0			PUT1_YEAST
2	LIFAGK	28.56	113	1	647.40	648.41	-0.42	1	0			RL402_YEAST...
3	LLFQK	21.38	113	3	647.40	648.41	-0.42	1	0			ARGR2_YEAST
4	LVDLIK	25.99	182	1	699.45	350.73	-0.28	2	0			PHSG_YEAST
5	LVDLLK	25.99	182	2	699.45	350.73	-0.28	2	0			BRR2_YEAST
6	LVDLIK	24.51	185	1	699.45	350.73	0.63	2	0			PHSG_YEAST
7	LVDLLK	24.51	185	2	699.45	350.73	0.63	2	0			BRR2_YEAST
8	LVDLIK	22.23	187	1	699.45	350.73	1.17	2	0			PHSG_YEAST
9	LVDLLK	22.23	187	2	699.45	350.73	1.17	2	0			BRR2_YEAST
10	VPFGGVK	31.28	200	1	702.41	352.21	0.24	2	0			ALDH6_YEAST
11	AAFIER	28.59	223	1	705.38	353.70	-0.37	2	0			RIR4_YEAST
12	AAFIER	23.53	225	1	705.38	353.70	1.47	2	0			RIR4_YEAST
13	LADFLK	24.19	227	1	705.41	353.71	-0.91	2	0			SYYC_YEAST
14	AIDLFK	25.18	230	1	705.41	353.71	2.06	2	0			SYRC_YEAST
15	AIDLFK	25.12	232	1	705.41	353.71	2.65	2	0			SYRC_YEAST
16	LADFLK	20.84	232	2	705.41	353.71	2.65	2	0			SYYC_YEAST
17	AGAFITK	34.03	239	1	706.40	354.21	-0.79	2	0			PYRD_YEAST
18	AGAFITK	41.53	240	1	706.40	354.21	0.20	2	0			PYRD_YEAST
19	KAMITK	27.74	240	2	706.40	354.21	-4.54	2	1		Oxidation (M3)	FMC1_YEAST
20	FGMDLK	34.44	256	1	709.35	355.68	-0.34	2	0			RIR4_YEAST
21	FGMDLK	23.01	258	1	709.35	355.68	0.61	2	0			RIR4_YEAST
22	FGMDLK	20.96	259	1	709.35	355.68	0.70	2	0			RIR4_YEAST
23	DHLLGR	24.18	264	1	709.39	355.70	-0.91	2	0			RL16B_YEAST
24	HILSIK	22.97	268	1	709.45	355.73	-0.69	2	0			PYR1_YEAST
25	HLISIK	22.97	268	2	709.45	355.73	-0.69	2	0			OTC_YEAST
26	HILSIK	23.16	269	1	709.45	355.73	-0.58	2	0			PYR1_YEAST
27	HLISIK	23.16	269	2	709.45	355.73	-0.58	2	0			OTC_YEAST
28	HLSLLK	21.12	269	3	709.45	355.73	-0.58	2	0			SKP2_YEAST
29	HILSIK	24.81	272	2	709.45	355.73	-0.18	2	0			PYR1_YEAST
30	HLISIK	24.81	272	3	709.45	355.73	-0.18	2	0			OTC_YEAST
31	HLSLLK	28.22	272	1	709.45	355.73	-0.18	2	0			SKP2_YEAST
32	HILSIK	30.48	274	2	709.45	355.73	0.13	2	0			PYR1_YEAST
33	HLISIK	30.48	274	3	709.45	355.73	0.13	2	0			OTC_YEAST
34	HLSLLK	32.31	274	1	709.45	355.73	0.13	2	0			SKP2_YEAST
35	HILSIK	25.34	275	2	709.45	355.73	0.60	2	0			PYR1_YEAST
36	HLISIK	25.34	275	3	709.45	355.73	0.60	2	0			OTC_YEAST
37	HLSLLK	26.93	275	1	709.45	355.73	0.60	2	0			SKP2_YEAST
38	HILSIK	28.43	276	1	709.45	355.73	0.80	2	0			PYR1_YEAST

Note : Abbreviations used are listed here

Peptides Window

If you click on Peptides sub-menu, you obtain this window:


Tasks Log
F067897 Peptides
Peptide
Score
Calc. Mass
Exp. MoZ
Ppm
Charge
Missed Cl.
Protein Set Count
RT
PTM
3726 LTGMAFR 33.60 810.41 406.21 -0.42 2 0 3 19.83 Oxidation (M4)
3727 LTGMAFR 30.99 794.41 398.21 -0.15 2 0 3 38.83
3728 LIFAGK 28.56 647.40 648.41 -0.42 1 0 3 33.34
3729 HIDAGAKK 28.52 838.47 420.24 0.46 2 1 3 6.56
3730 IINEPTAAAIAYG... 120.71 1786.98 894.50 0.18 2 1 4 69.65
3731 IINEPTAAAIAYG... 119.70 1658.89 830.45 1.01 2 0 4 79.47
3732 DAGTIAGLNVLR 88.22 1198.67 600.34 0.68 2 0 4 73.05
3733 FEELCADLFR 71.29 1298.60 650.31 0.72 2 0 4 82.55 Carbamidometh...
3734 ARFEELCADLFR 56.80 1525.73 763.88 1.27 2 1 4 83.68 Carbamidometh...
3735 TKDNNLLGK 41.66 1001.55 501.78 -0.61 2 1 4 13.70
3736 FELSGIPPAPR 30.34 1182.64 592.33 -0.14 2 0 4 69.89
Protein Set
Description
Score
Proteins
Peptides
Peptide Match Count
Specific Peptide Match Count
Unique Seq. Count
1 RS27A_YEAST sp|P05759|RS27A... 539.76 2 (1, 1) 8 73 5 8
2 RL402_YEAST sp|P0CH09|RL402... 417.63 3 (2, 1) 7 73 5 7
3 RS27A_HUMAN P62979ups|RS27A... 306.42 1 (1, 0) 6 29 1 6
Typical Protein:
ATCC 204508 / S288c) GN=RPS31 PE=1 SV=3
Protein
De...
Sam...
Score
Pepti...
Uni...
Mass
RS2... sp|P... 539.76 8 8 172...
UBI... sp|P... 383.96 6 6 428...
Prev. AA
Peptide
Next AA
Score
Protein S. Matches
Start
Stop
Cal...
Ex...
Ppm
C...
Mi...
RT
K TITLEVESS... S 112.95 2 (RS27A_YEAST,R... 12 27 176... 882... 0.68 2 0 13...
R ESNPTGAG D 104.86 1 (RS27A_YEAST) 120 136 186... 931... -0.43 2 0 45...
K IQDKEGIP... L 91.71 3 (RS27A_YEAST,R... 30 42 152... 762... -0.86 2 1 14...
K ESTLHLVLR L 66.97 3 (RS27A_YEAST,R... 64 72 106... 534... -0.52 2 0 56...
K LAVLSYYK V 50.94 1 (RS27A_YEAST) 100 107 955.54 478... 0.72 2 0 58...
- MQIFVK T 46.47 3 (RS27A_YEAST,R... 1 6 764.43 383... 0.25 2 0 45...
R TLSDYNIQK E 37.30 3 (RS27A_YEAST,R... 55 63 108... 541... -0.77 2 0 28...
R LIFAGK Q 28.56 3 (RS27A_YEAST,R... 43 48 647.40 648... -0.42 1 0 33...

Upper View: list of all Peptides

Middle View: list of all Protein Sets containing the selected peptide.

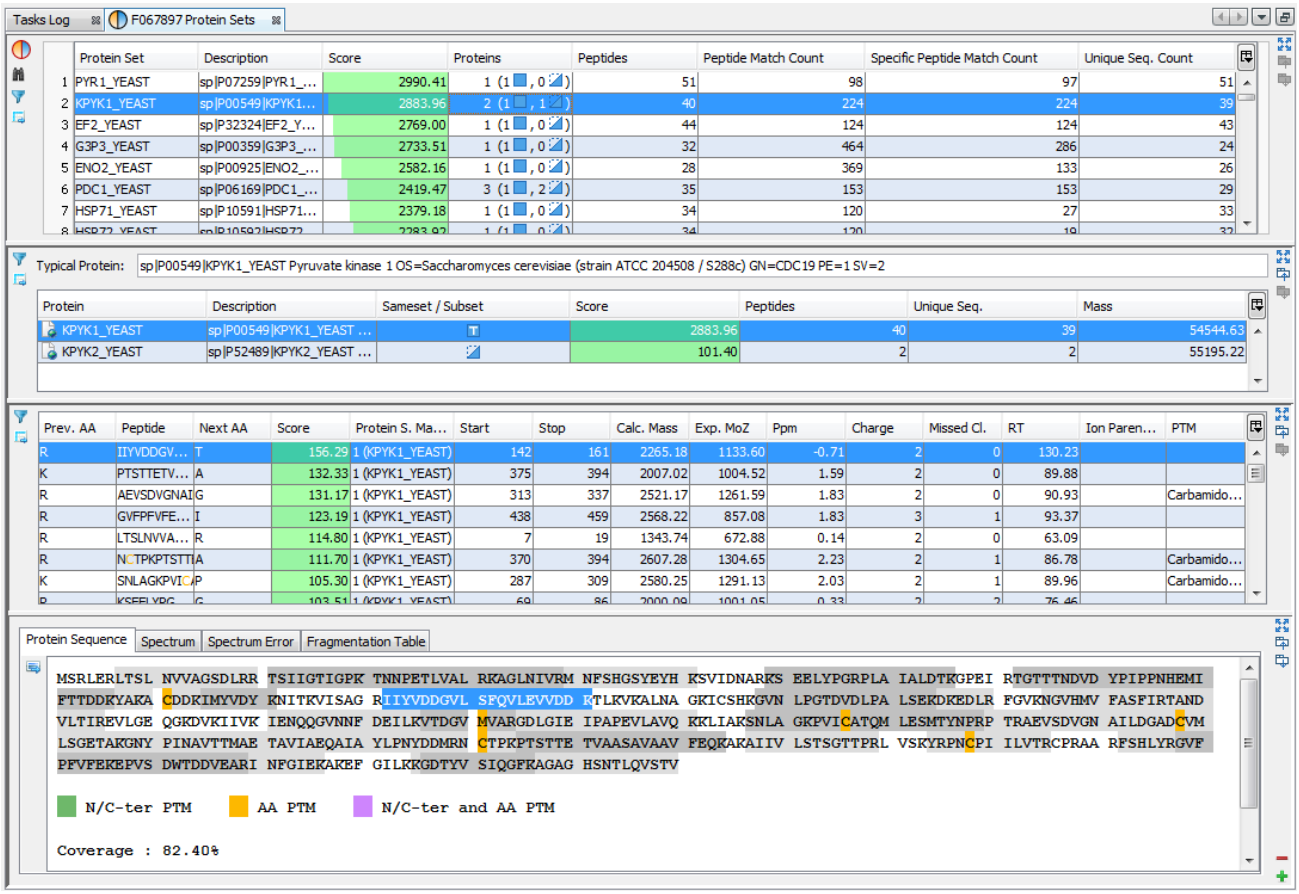
Bottom Left View: list of all Proteins of the selected Protein Set

Bottom Right View: list of all Peptides of the selected Protein

Note: Abbreviations used are listed here

Protein Sets Window

If you click on Protein Sets sub-menu, you obtain this window:


Tasks Log
F067897 Protein Sets
Protein Set | Description | Score | Proteins | Peptides | Peptide Match Count | Specific Peptide Match Count | Unique Seq. Count
1 PYR1_YEAST | sp|P07259|PYR1_... | 2990.41 | 1 (1 , 0) | 51 | 98 | 97 | 51
2 KPYK1_YEAST | sp|P00549|KPYK1... | 2883.96 | 2 (1 , 1) | 40 | 224 | 224 | 39
3 EF2_YEAST | sp|P32324|EF2_Y... | 2769.00 | 1 (1 , 0) | 44 | 124 | 124 | 43
4 G3P3_YEAST | sp|P00359|G3P3_... | 2733.51 | 1 (1 , 0) | 32 | 464 | 286 | 24
5 ENO2_YEAST | sp|P00925|ENO2_... | 2582.16 | 1 (1 , 0) | 28 | 369 | 133 | 26
6 PDC1_YEAST | sp|P06169|PDC1_... | 2419.47 | 3 (1 , 2) | 35 | 153 | 153 | 29
7 HSP71_YEAST | sp|P10591|HSP71... | 2379.18 | 1 (1 , 0) | 34 | 120 | 27 | 33
Typical Protein: sp|P00549|KPYK1_YEAST Pyruvate kinase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) GN=CDC19 PE=1 SV=2
Protein | Description | Sameset / Subset | Score | Peptides | Unique Seq. | Mass
KPYK1_YEAST | sp|P00549|KPYK1_YEAST ... | T | 2883.96 | 40 | 39 | 54544.63
KPYK2_YEAST | sp|P52489|KPYK2_YEAST ... | | 101.40 | 2 | 2 | 55195.22
Prev. AA | Peptide | Next AA | Score | Protein S. Ma... | Start | Stop | Calc. Mass | Exp. MoZ | Ppm | Charge | Missed Cl. | RT | Ion Paren... | PTM
R | IIYVDDGV... | T | 156.29 | 1 (KPYK1_YEAST) | 142 | 161 | 2265.18 | 1133.60 | -0.71 | 2 | 0 | 130.23
K | PTSTTETV... | A | 132.33 | 1 (KPYK1_YEAST) | 375 | 394 | 2007.02 | 1004.52 | 1.59 | 2 | 0 | 89.88
R | AEVSDVGNAI | G | 131.17 | 1 (KPYK1_YEAST) | 313 | 337 | 2521.17 | 1261.59 | 1.83 | 2 | 0 | 90.93 | Carbamido...
R | GVFPFVFE... | I | 123.19 | 1 (KPYK1_YEAST) | 438 | 459 | 2568.22 | 857.08 | 1.83 | 3 | 1 | 93.37
R | LTSLNVVA... | R | 114.80 | 1 (KPYK1_YEAST) | 7 | 19 | 1343.74 | 672.88 | 0.14 | 2 | 0 | 63.09
R | NCTPKPTSTTI | A | 111.70 | 1 (KPYK1_YEAST) | 370 | 394 | 2607.28 | 1304.65 | 2.23 | 2 | 1 | 86.78 | Carbamido...
K | SNLAGKPVIC | P | 105.30 | 1 (KPYK1_YEAST) | 287 | 309 | 2580.25 | 1291.13 | 2.03 | 2 | 1 | 89.96 | Carbamido...
Protein Sequence
Spectrum
Spectrum Error
Fragmentation Table
MSRLERLTSL NVVAGSDLRR TSIIGTIGPK TNNPETLVAL RKAGLNIVRM NFSHGSYEYH KSVIDNARKS EELYPGRPLA IALDTKGPEI RTGTTTNDVD YPIPPNHEMI
FTTDDKYAKA CDDKIMYVDY KNITKVISAG RIIYVDDGVL SFQVLEVVDD KTLKVKALNA GKICSHKGVN LPGTDVDLPA LSEKDKEDLR FGVKNGVHMV FASFIRTAND
VLTIREVLGE QGKDVKIIVK IENQQGVNNF DEILKVTDGV MVARGDLGIE IPAPEVLAVQ KKLIAKSNLA GKPVICATQM LESMTYNPRP TRAEVSDVGN AILDGADCVM
LSGETAKGNY PINAVTTMAE TAVIAEQAIA YLPNYDDMRN CTPRPTSTTE TVAASAVAAV FEQKAKAIIV LSTSGTTPRL VSKYRPNCPI ILVTRCPRAA RFSHLYRGVF
PFVFEKEPVS DWTDDVEARI NFGIEKAKEF GILKKGDTYV SIQGFKAGAG HSNTLQVSTV
N/C-ter PTM
AA PTM
N/C-ter and AA PTM
Coverage : 82.40%

View 1 (at the top): list of all Protein Sets

Note: In the column Proteins, 8 (2,6) means that there are 8 proteins in the Protein Set. 2 in the sameset, 6 in the subset.

View 2: list of all Proteins of the selected Protein Set.

View 3: list of all Peptides of the selected Protein

View 4: Protein Sequence of the previously selected Protein and Spectrum of the selected Peptide.

Note: Abbreviations used are listed here

Display Additional Informations on Search Result/Identification Summary

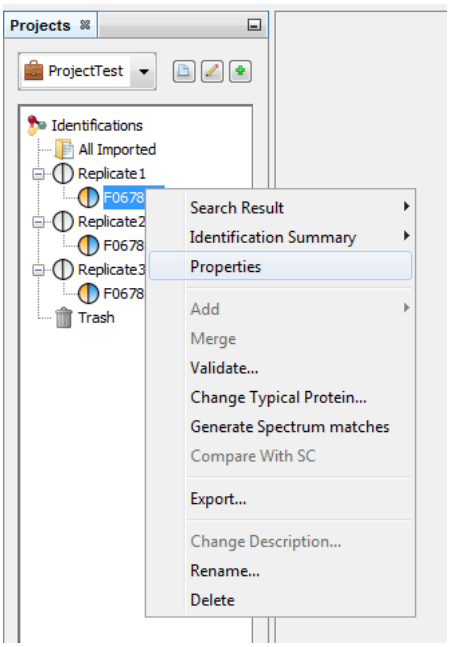
Functionality Access

To display properties of a Search Result/Identification Summary:

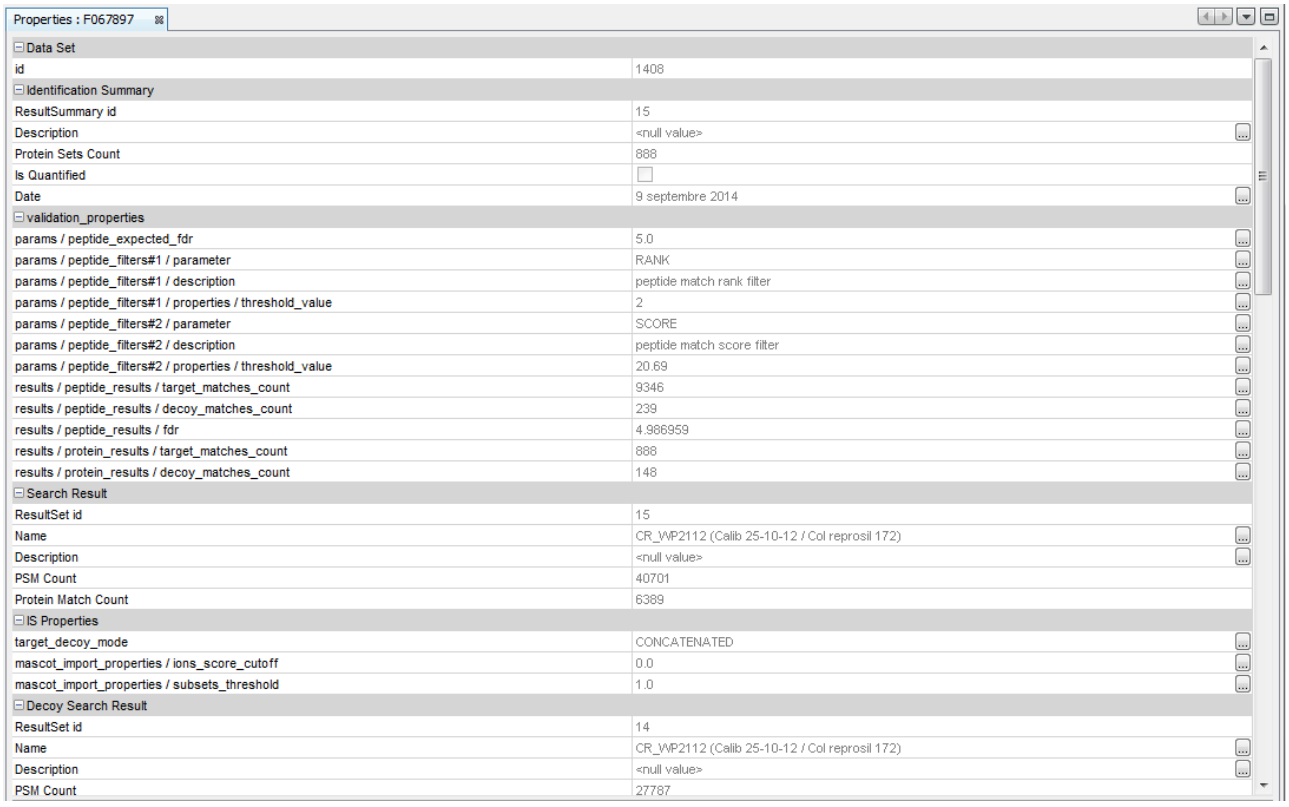
- right click on a Search Result/Identification Summary

- click on the menu “Properties”

Note: it is possible to select multiple Search Results/Identification Summaries to compare the values.

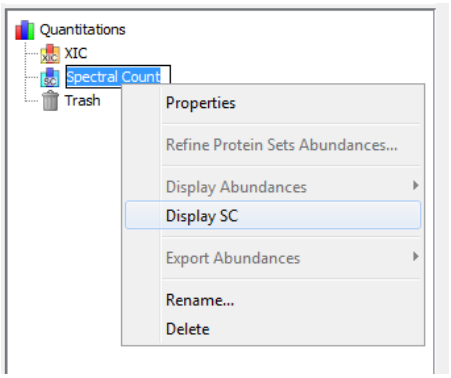


Properties Window

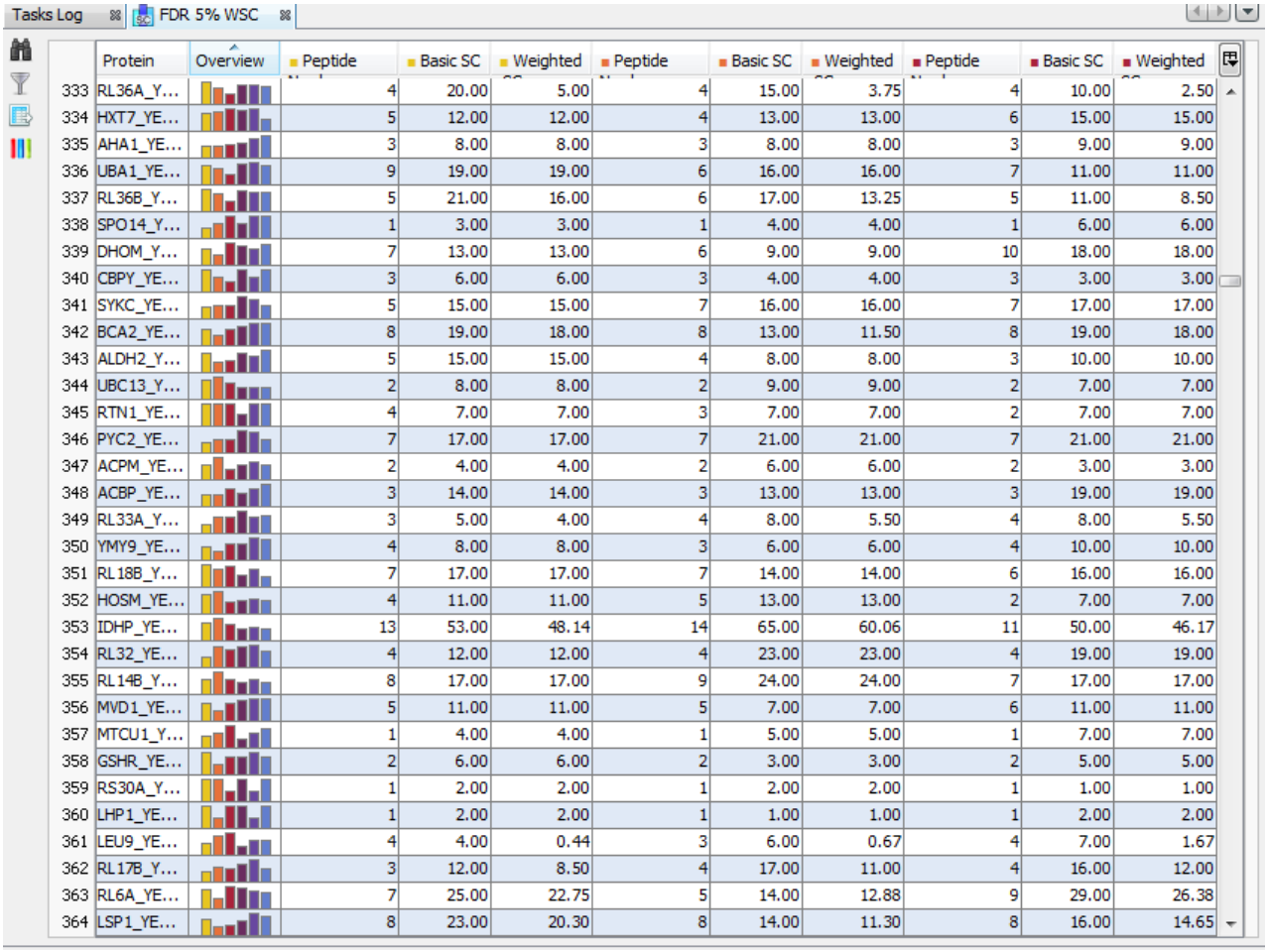

Properties : F067897
Data Set
id 1408
Identification Summary
ResultSummary id 15
Description <null value>
Protein Sets Count 888
Is Quantified
Date 9 septembre 2014
validation_properties
params / peptide_expected_fdr 5.0
params / peptide_filters#1 / parameter RANK
params / peptide_filters#1 / description peptide match rank filter
params / peptide_filters#1 / properties / threshold_value 2
params / peptide_filters#2 / parameter SCORE
params / peptide_filters#2 / description peptide match score filter
params / peptide_filters#2 / properties / threshold_value 20.69
results / peptide_results / target_matches_count 9346
results / peptide_results / decoy_matches_count 239
results / peptide_results / fdr 4.986959
results / protein_results / target_matches_count 888
results / protein_results / decoy_matches_count 148
Search Result
ResultSet id 15
Name CR_WP2112 (Calib 25-10-12 / Col reprosil 172)
Description <null value>
PSM Count 40701
Protein Match Count 6389
IS Properties
target_decoy_mode CONCATENATED
mascot_import_properties / ions_score_cutoff 0.0
mascot_import_properties / subsets_threshold 1.0
Decoy Search Result
ResultSet id 14
Name CR_WP2112 (Calib 25-10-12 / Col reprosil 172)
Description <null value>
PSM Count 27787

Display a Spectral Count

You can display a generated Spectral Count by using the right mouse popup.



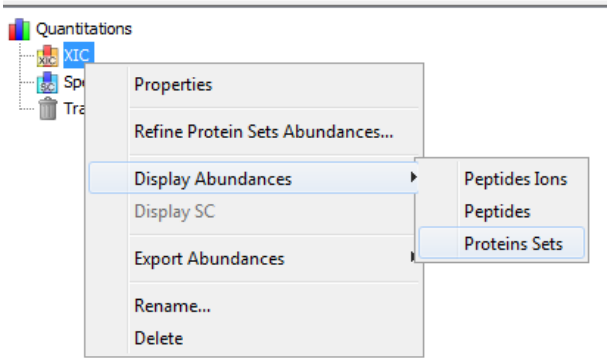
To have more details about the results, see spectral count result



	Protein	Overview	Peptide	Basic SC	Weighted	Peptide	Basic SC	Weighted	Peptide	Basic SC	Weighted
333	RL36A_Y...		4	20.00	5.00	4	15.00	3.75	4	10.00	2.50
334	HXT7_YE...		5	12.00	12.00	4	13.00	13.00	6	15.00	15.00
335	AHA1_YE...		3	8.00	8.00	3	8.00	8.00	3	9.00	9.00
336	UBA1_YE...		9	19.00	19.00	6	16.00	16.00	7	11.00	11.00
337	RL36B_Y...		5	21.00	16.00	6	17.00	13.25	5	11.00	8.50
338	SPO14_Y...		1	3.00	3.00	1	4.00	4.00	1	6.00	6.00
339	DHOM_Y...		7	13.00	13.00	6	9.00	9.00	10	18.00	18.00
340	CBPY_YE...		3	6.00	6.00	3	4.00	4.00	3	3.00	3.00
341	SYKC_YE...		5	15.00	15.00	7	16.00	16.00	7	17.00	17.00
342	BCA2_YE...		8	19.00	18.00	8	13.00	11.50	8	19.00	18.00
343	ALDH2_Y...		5	15.00	15.00	4	8.00	8.00	3	10.00	10.00
344	UBC13_Y...		2	8.00	8.00	2	9.00	9.00	2	7.00	7.00
345	RTN1_YE...		4	7.00	7.00	3	7.00	7.00	2	7.00	7.00
346	PYC2_YE...		7	17.00	17.00	7	21.00	21.00	7	21.00	21.00
347	ACPM_YE...		2	4.00	4.00	2	6.00	6.00	2	3.00	3.00
348	ACBP_YE...		3	14.00	14.00	3	13.00	13.00	3	19.00	19.00
349	RL33A_Y...		3	5.00	4.00	4	8.00	5.50	4	8.00	5.50
350	YMY9_YE...		4	8.00	8.00	3	6.00	6.00	4	10.00	10.00
351	RL18B_Y...		7	17.00	17.00	7	14.00	14.00	6	16.00	16.00
352	HOSM_YE...		4	11.00	11.00	5	13.00	13.00	2	7.00	7.00
353	IDHP_YE...		13	53.00	48.14	14	65.00	60.06	11	50.00	46.17
354	RL32_YE...		4	12.00	12.00	4	23.00	23.00	4	19.00	19.00
355	RL14B_Y...		8	17.00	17.00	9	24.00	24.00	7	17.00	17.00
356	MVD1_YE...		5	11.00	11.00	5	7.00	7.00	6	11.00	11.00
357	MTCU1_Y...		1	4.00	4.00	1	5.00	5.00	1	7.00	7.00
358	GSHR_YE...		2	6.00	6.00	2	3.00	3.00	2	5.00	5.00
359	RS30A_Y...		1	2.00	2.00	1	2.00	2.00	1	1.00	1.00
360	LHP1_YE...		1	2.00	2.00	1	1.00	1.00	1	2.00	2.00
361	LEU9_YE...		4	4.00	0.44	3	6.00	0.67	4	7.00	1.67
362	RL17B_Y...		3	12.00	8.50	4	17.00	11.00	4	16.00	12.00
363	RL6A_YE...		7	25.00	22.75	5	14.00	12.88	9	29.00	26.38
364	LSP1_YE...		8	23.00	20.30	8	14.00	11.30	8	16.00	14.65

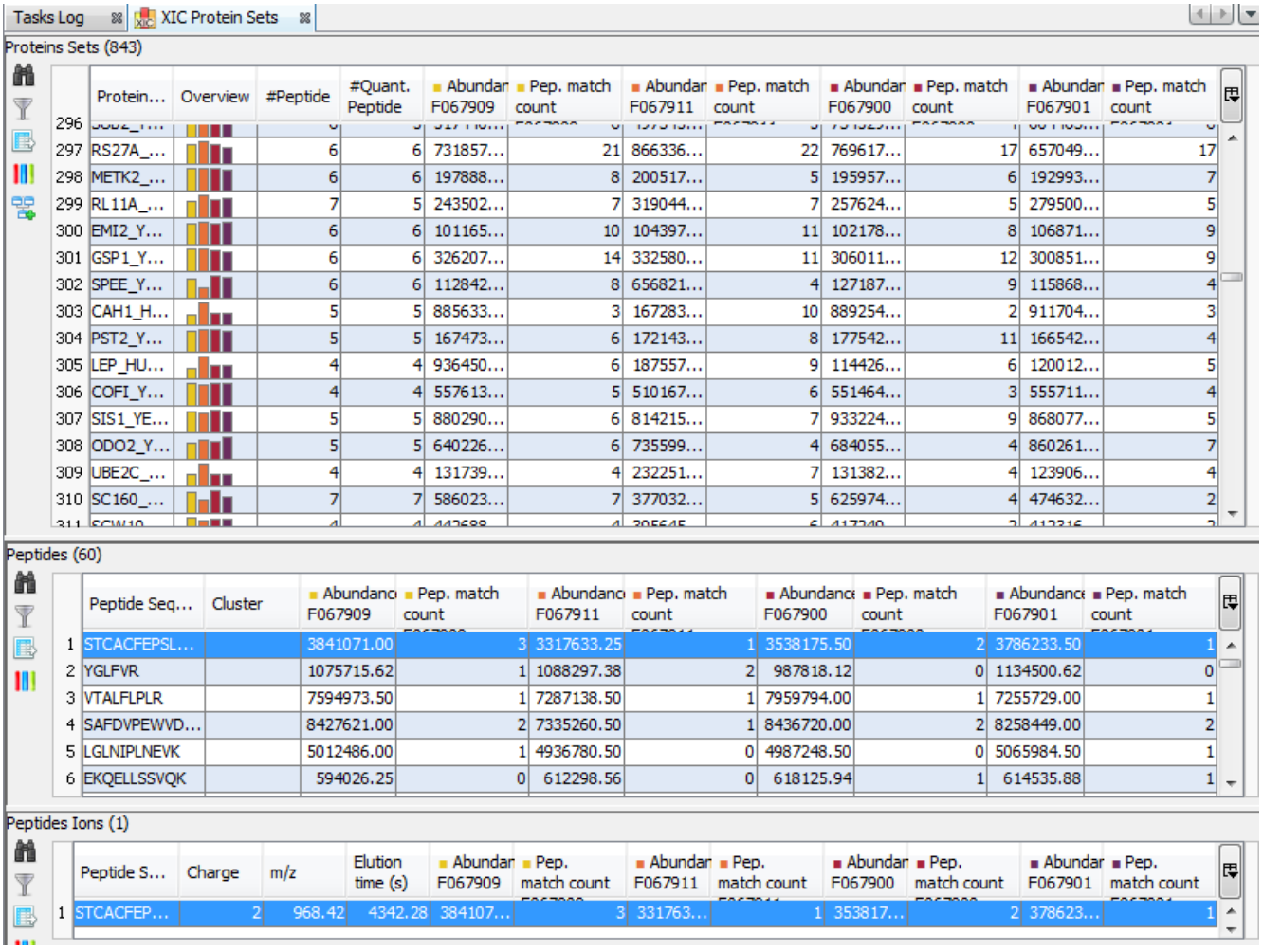
Display a XIC

To display a XIC, right click on the selected XIC node in the Quantitation tree, and select “Display Abundances”, and then the level you want to display:



Display Protein Sets

By clicking on “Display Abundances” / “Protein Sets”, you can see all quantified protein sets. For each quantified protein set, you can see below all peptides linked to the selected protein set and peptides Ions linked to the selected peptide



The overview is based on the abundances values.

For each quantitation channel, are displayed:

- The raw abundance

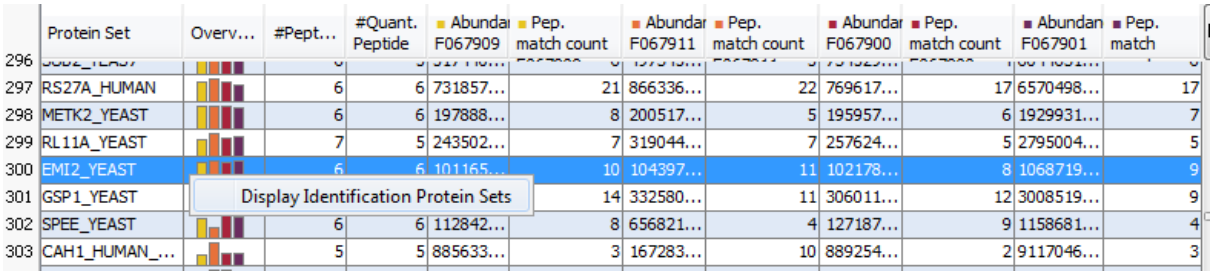
- The peptide match count (by default)

- The abundance (by default)

- The selection level

By clicking on the “Column Display Button” , you can choose the information you want to display.

To display the identification protein set view, click right on the selected protein Set and select “Display Identification Protein Sets” menu in the popup.



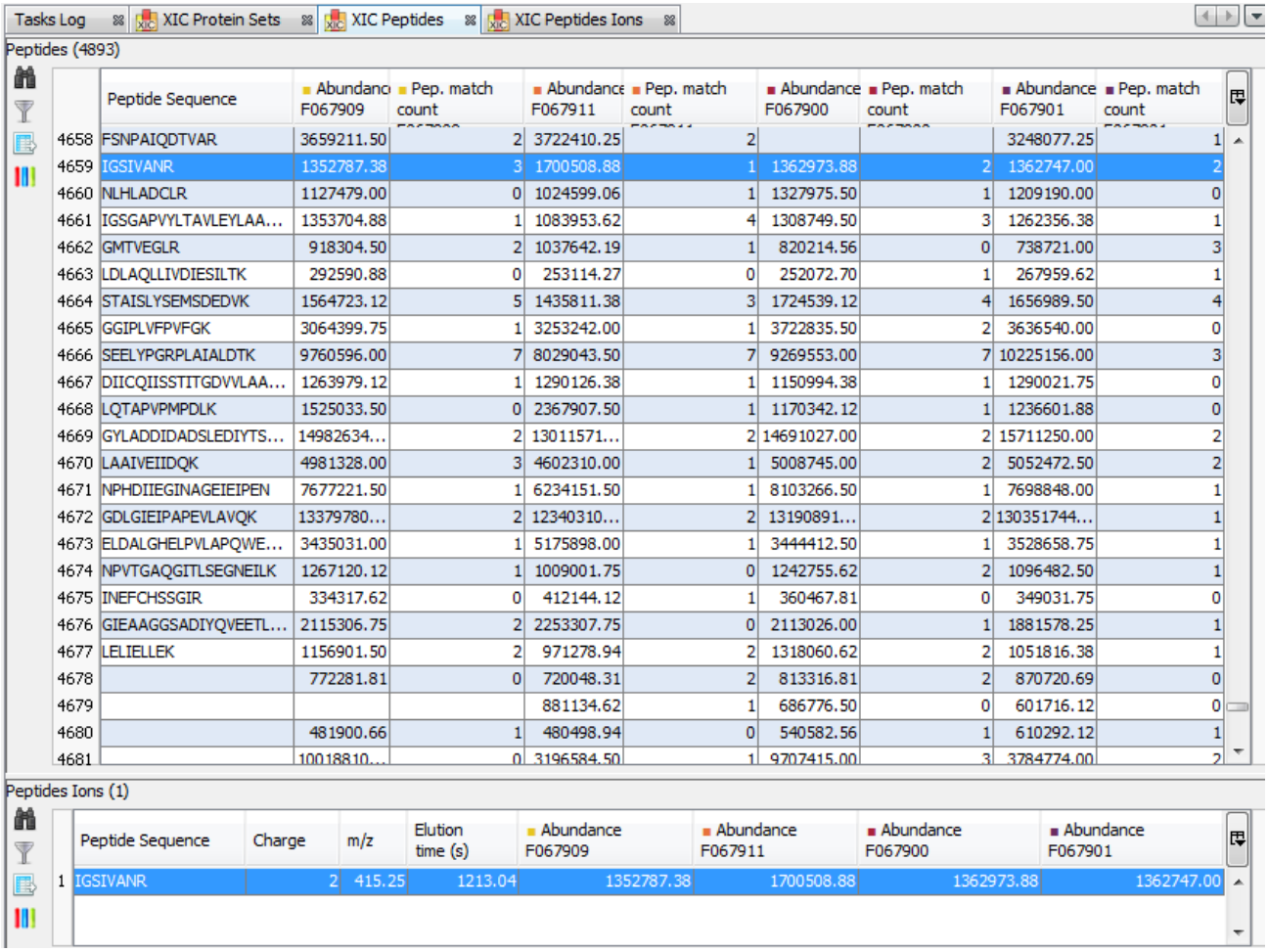
Display peptides

By clicking on “Display Abundances “/ “Peptides”, you can see:

- identified and quantified Peptides

- non identified but quantified peptides

- identified but not quantified peptides (linked to a quantified protein)

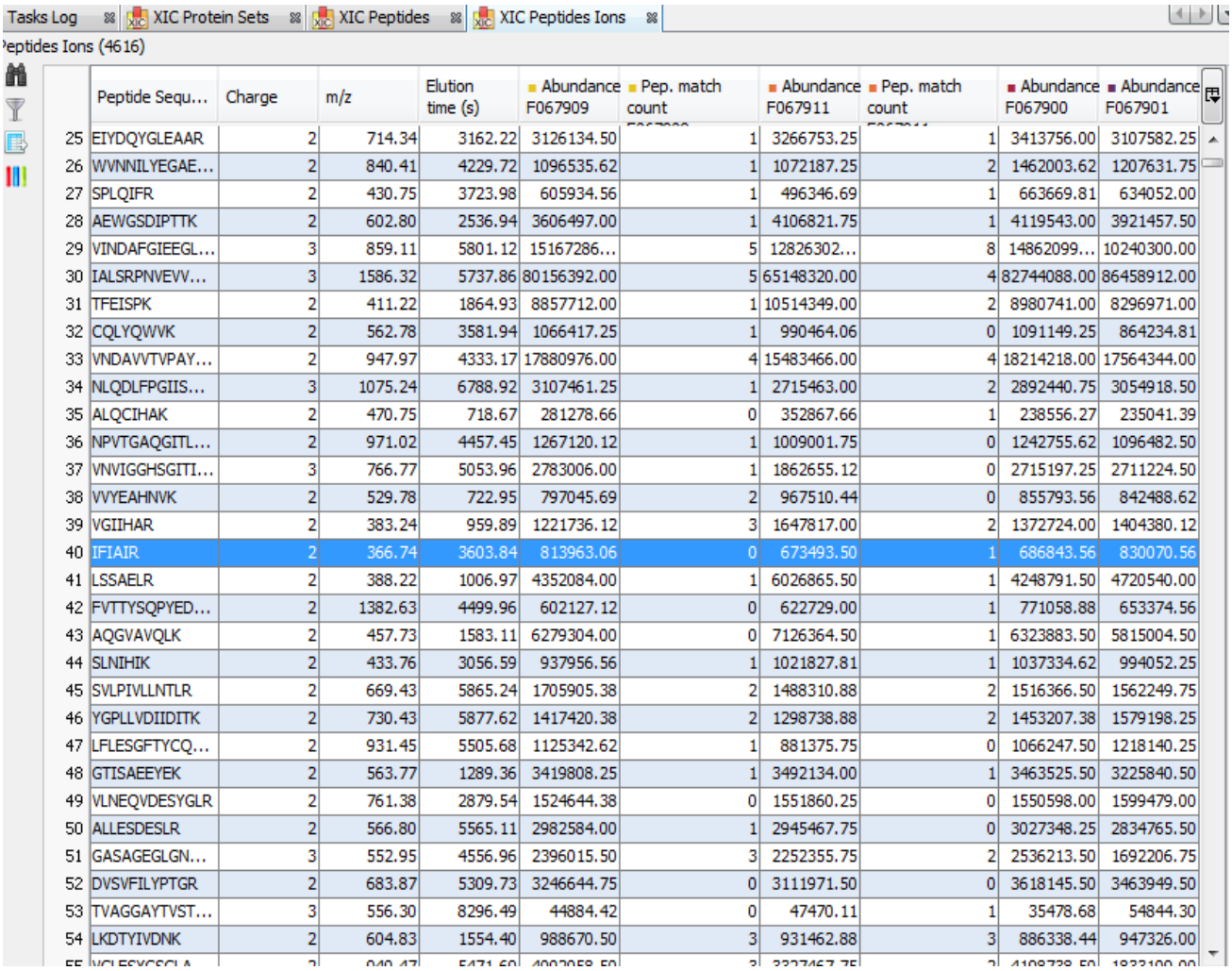


Display Peptides Ions

By clicking on “Display Abundances” / “Peptides Ions”, you can see:

- all identified and quantified Peptides Ions

- non identified but quantified peptides Ions

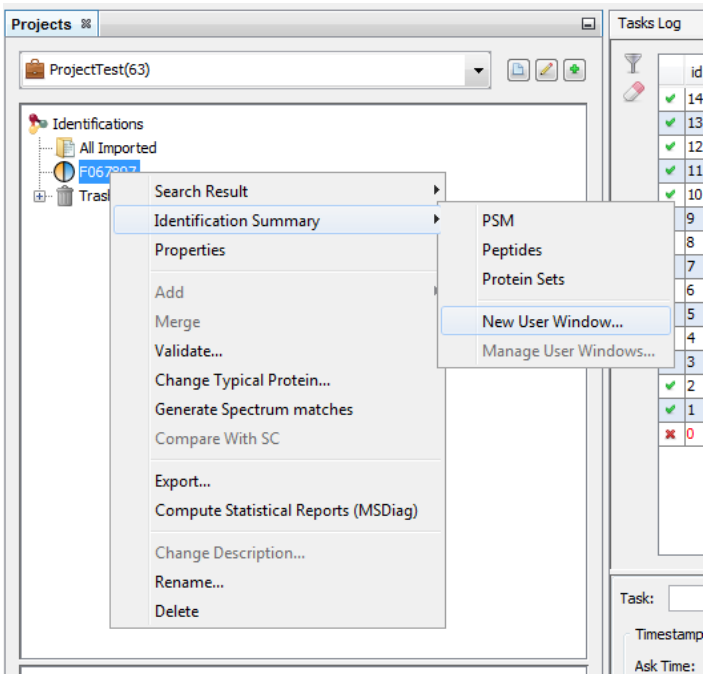


	Peptide Sequ...	Charge	m/z	Elution time (s)	Abundance F067909	Pep. match count	Abundance F067911	Pep. match count	Abundance F067900	Abundance F067901
25	EIYDQYGLEAAR	2	714.34	3162.22	3126134.50	1	3266753.25	1	3413756.00	3107582.25
26	WVNNILYEGAE...	2	840.41	4229.72	1096535.62	1	1072187.25	2	1462003.62	1207631.75
27	SPLQIFR	2	430.75	3723.98	605934.56	1	496346.69	1	663669.81	634052.00
28	AEWGSDIPTTK	2	602.80	2536.94	3606497.00	1	4106821.75	1	4119543.00	3921457.50
29	VINDAFGIEEGL...	3	859.11	5801.12	15167286...	5	12826302...	8	14862099...	10240300.00
30	IALSRPNVEVV...	3	1586.32	5737.86	80156392.00	5	65148320.00	4	82744088.00	86458912.00
31	TFEISPK	2	411.22	1864.93	8857712.00	1	10514349.00	2	8980741.00	8296971.00
32	CQLYQWVK	2	562.78	3581.94	1066417.25	1	990464.06	0	1091149.25	864234.81
33	VNDAVVTVPAY...	2	947.97	4333.17	17880976.00	4	15483466.00	4	18214218.00	17564344.00
34	NLQDLFPGIIS...	3	1075.24	6788.92	3107461.25	1	2715463.00	2	2892440.75	3054918.50
35	ALQCIHAK	2	470.75	718.67	281278.66	0	352867.66	1	238556.27	235041.39
36	NPVTGAQGITL...	2	971.02	4457.45	1267120.12	1	1009001.75	0	1242755.62	1096482.50
37	VNVIGGHSGITI...	3	766.77	5053.96	2783006.00	1	1862655.12	0	2715197.25	2711224.50
38	VVYEAHNVK	2	529.78	722.95	797045.69	2	967510.44	0	855793.56	842488.62
39	VGIIHAR	2	383.24	959.89	1221736.12	3	1647817.00	2	1372724.00	1404380.12
40	IFIAIR	2	366.74	3603.84	813963.06	0	673493.50	1	686843.56	830070.56
41	LSSAELR	2	388.22	1006.97	4352084.00	1	6026865.50	1	4248791.50	4720540.00
42	FVTTYSQPYED...	2	1382.63	4499.96	602127.12	0	622729.00	1	771058.88	653374.56
43	AQGVAVQLK	2	457.73	1583.11	6279304.00	0	7126364.50	1	6323883.50	5815004.50
44	SLNIHIK	2	433.76	3056.59	937956.56	1	1021827.81	1	1037334.62	994052.25
45	SVLPIVLLNTLR	2	669.43	5865.24	1705905.38	2	1488310.88	2	1516366.50	1562249.75
46	YGPLLVDIIDITK	2	730.43	5877.62	1417420.38	2	1298738.88	2	1453207.38	1579198.25
47	LFLESGFTYCQ...	2	931.45	5505.68	1125342.62	1	881375.75	0	1066247.50	1218140.25
48	GTISAEEYEK	2	563.77	1289.36	3419808.25	1	3492134.00	1	3463525.50	3225840.50
49	VLNEQVDESYGLR	2	761.38	2879.54	1524644.38	0	1551860.25	0	1550598.00	1599479.00
50	ALLESDESLR	2	566.80	5565.11	2982584.00	1	2945467.75	0	3027348.25	2834765.50
51	GASAGEGLGN...	3	552.95	4556.96	2396015.50	3	2252355.75	2	2536213.50	1692206.75
52	DVSVFILYPTGR	2	683.87	5309.73	3246644.75	0	3111971.50	0	3618145.50	3463949.50
53	TVAGGAYTVST...	3	556.30	8296.49	44884.42	0	47470.11	1	35478.68	54844.30
54	LKDTYIVDNK	2	604.83	1554.40	988670.50	3	931462.88	3	886338.44	947326.00

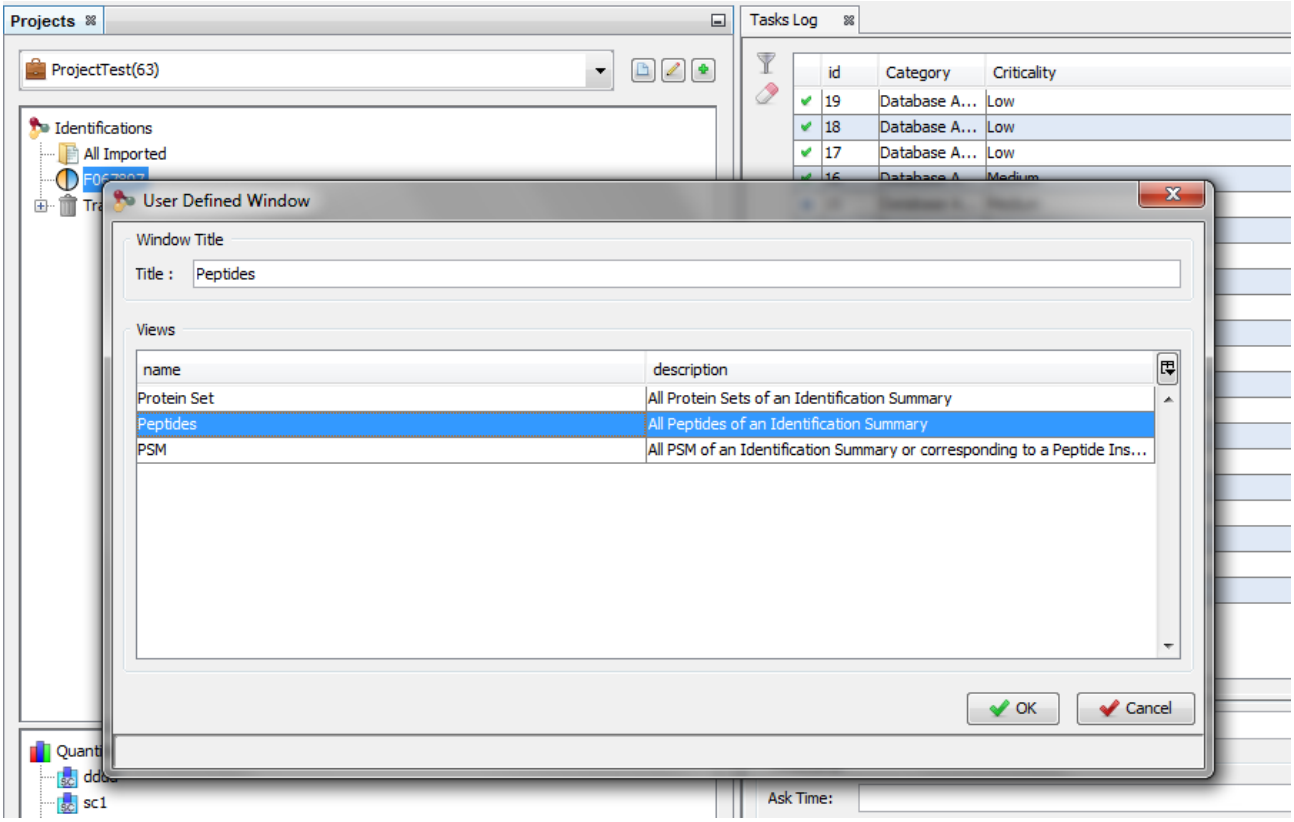
Create a User Window

You can lay out your own user window with the desired views.

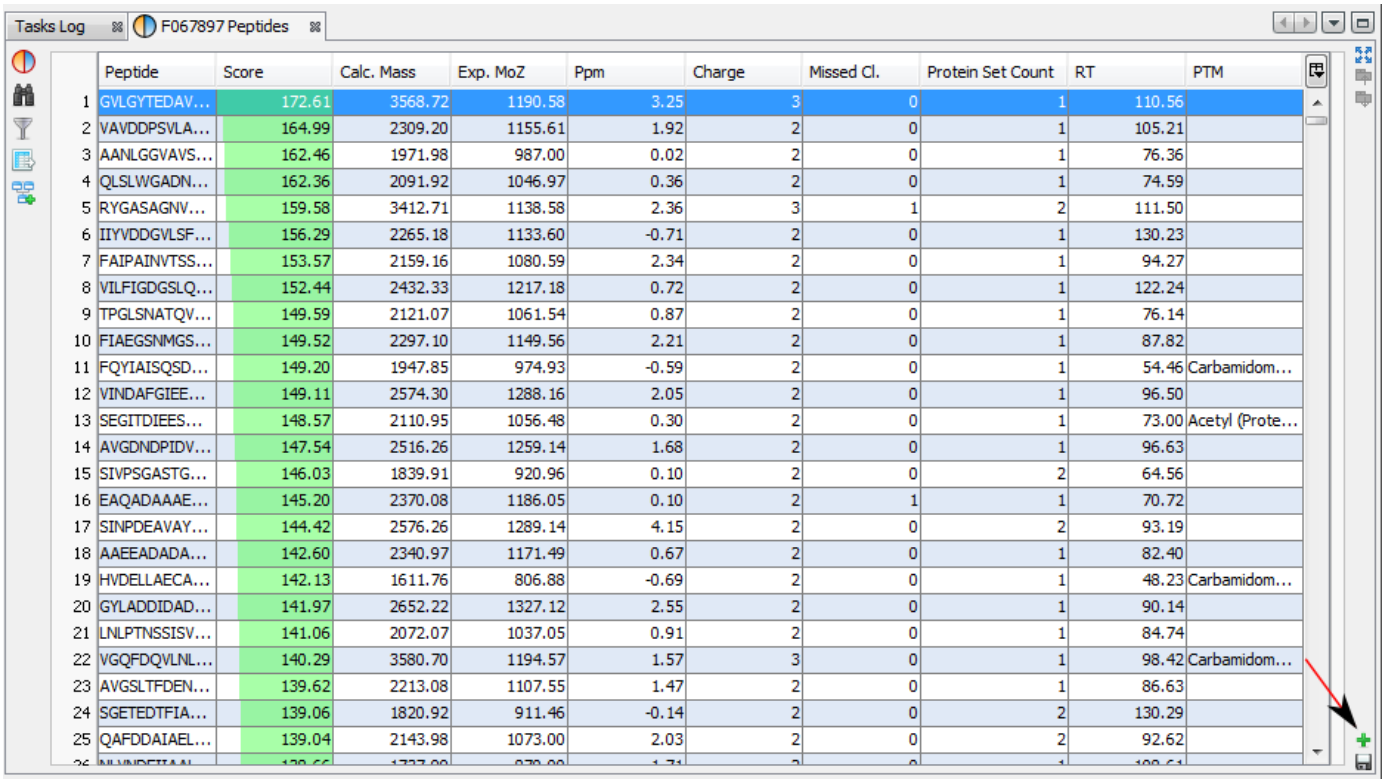
You can do it from an already displayed window or by using the right click mouse popup on a dataset like in the following example (Use menu “Search Result>New User Window…” or “Identification Summary>New User Window…”)



In the example, the user has clicked on “Identification Summary>New User Window…” and selects the Peptides View as the first view of his window.

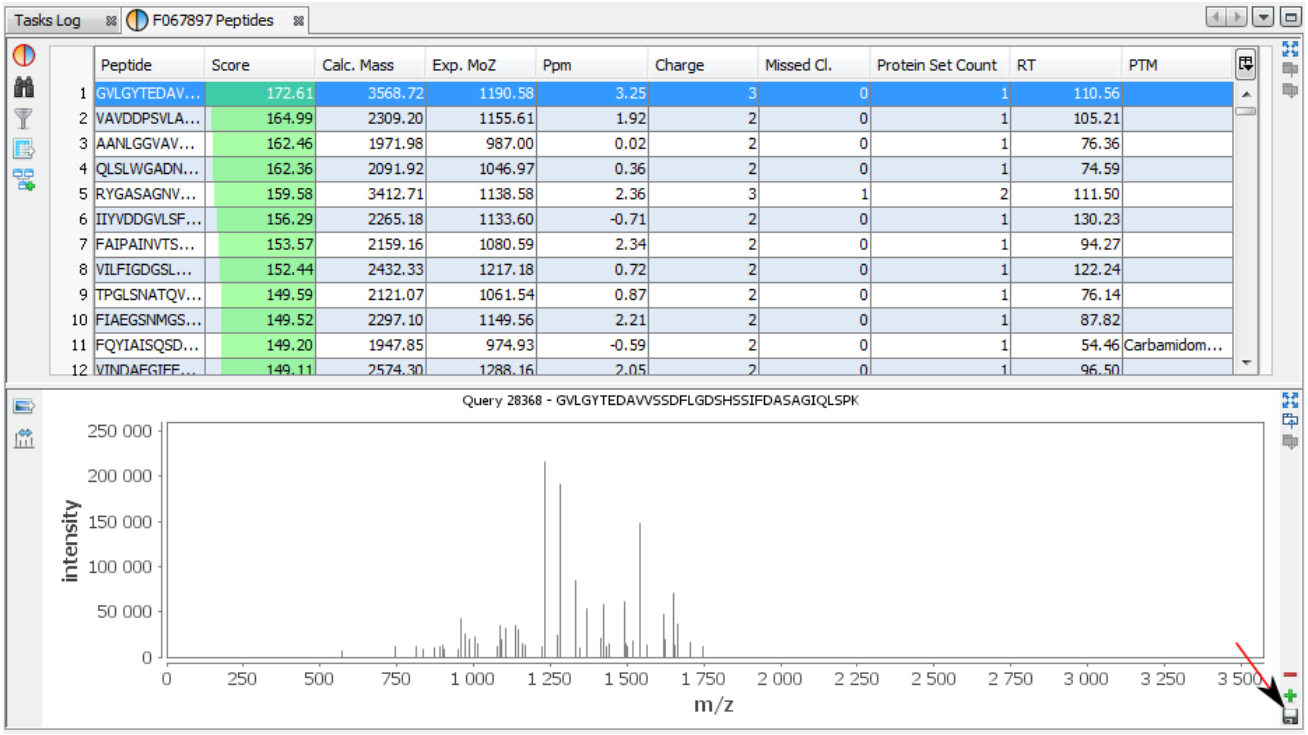


You can add other views by using the '+' button.



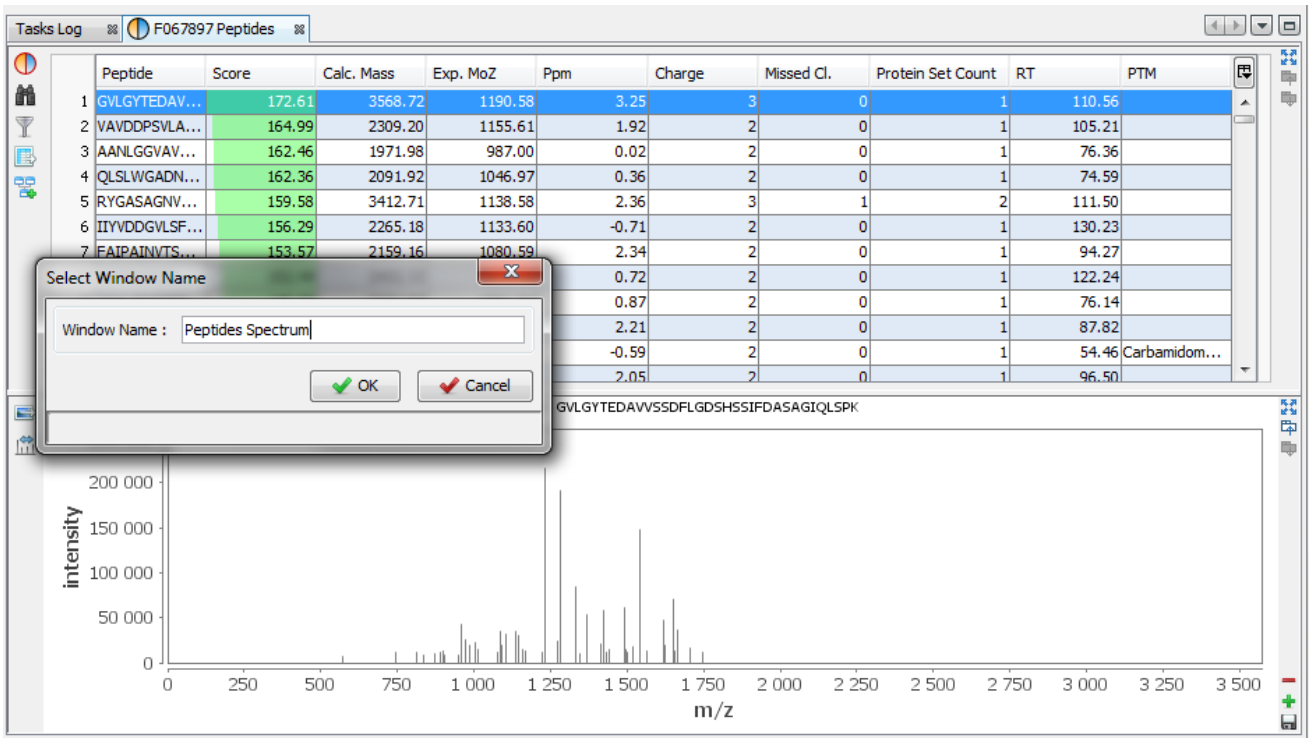
	Peptide	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Protein Set Count	RT	PTM
1	GVLGYTEDAV...	172.61	3568.72	1190.58	3.25	3	0	1	110.56	
2	VAVDDPSVLA...	164.99	2309.20	1155.61	1.92	2	0	1	105.21	
3	AANLGGVAVS...	162.46	1971.98	987.00	0.02	2	0	1	76.36	
4	QLSLWGADN...	162.36	2091.92	1046.97	0.36	2	0	1	74.59	
5	RYGASAGNV...	159.58	3412.71	1138.58	2.36	3	1	2	111.50	
6	IIYVDDGVLSF...	156.29	2265.18	1133.60	-0.71	2	0	1	130.23	
7	FAIPAINVTSS...	153.57	2159.16	1080.59	2.34	2	0	1	94.27	
8	VILFIGDGSLQ...	152.44	2432.33	1217.18	0.72	2	0	1	122.24	
9	TPGLSNATQV...	149.59	2121.07	1061.54	0.87	2	0	1	76.14	
10	FIAEGSNMGS...	149.52	2297.10	1149.56	2.21	2	0	1	87.82	
11	FQYIAISQSD...	149.20	1947.85	974.93	-0.59	2	0	1	54.46	Carbamidom...
12	VINDAFGIEE...	149.11	2574.30	1288.16	2.05	2	0	1	96.50	
13	SEGITDIEES...	148.57	2110.95	1056.48	0.30	2	0	1	73.00	Acetyl (Prote...
14	AVGDNDPIDV...	147.54	2516.26	1259.14	1.68	2	0	1	96.63	
15	SIVPSGASTG...	146.03	1839.91	920.96	0.10	2	0	2	64.56	
16	EAQADAAAE...	145.20	2370.08	1186.05	0.10	2	1	1	70.72	
17	SINPDEAVAY...	144.42	2576.26	1289.14	4.15	2	0	2	93.19	
18	AAEEADADA...	142.60	2340.97	1171.49	0.67	2	0	1	82.40	
19	HVDELLAECA...	142.13	1611.76	806.88	-0.69	2	0	1	48.23	Carbamidom...
20	GYLADDIDAD...	141.97	2652.22	1327.12	2.55	2	0	1	90.14	
21	LNLPTNSSISV...	141.06	2072.07	1037.05	0.91	2	0	1	84.74	
22	VGQFDQVLNL...	140.29	3580.70	1194.57	1.57	3	0	1	98.42	Carbamidom...
23	AVGSLTFDEN...	139.62	2213.08	1107.55	1.47	2	0	1	86.63	
24	SGETEDTFIA...	139.06	1820.92	911.46	-0.14	2	0	2	130.29	
25	QAFDDAIAEL...	139.04	2143.98	1073.00	2.03	2	0	2	92.62	

In this example, the user has added a Spectrum View and he saves his window by clicking on the “Disk” Button.

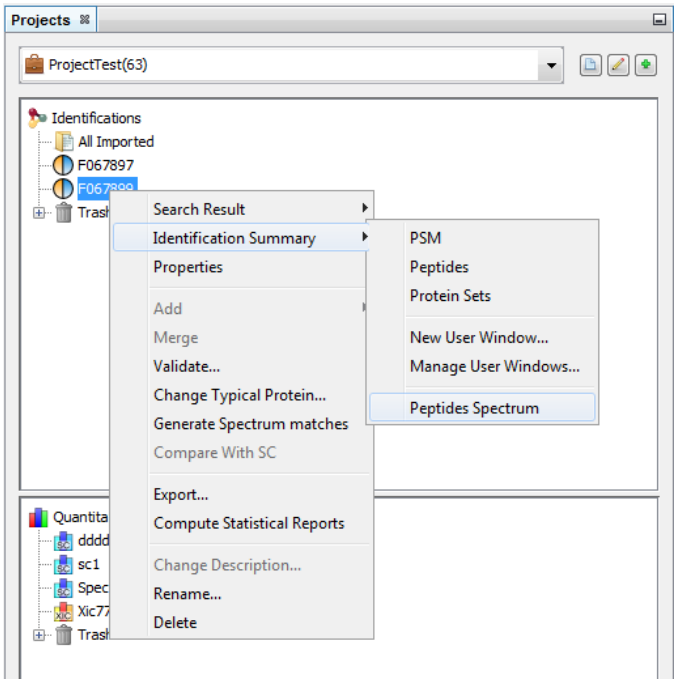


	Peptide	Score	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Protein Set Count	RT	PTM
1	GVLGYTEDAV...	172.61	3568.72	1190.58	3.25	3	0	1	110.56	
2	VAVDDPSVLA...	164.99	2309.20	1155.61	1.92	2	0	1	105.21	
3	AANLGGVAV...	162.46	1971.98	987.00	0.02	2	0	1	76.36	
4	QLSLWGADN...	162.36	2091.92	1046.97	0.36	2	0	1	74.59	
5	RYGASAGNV...	159.58	3412.71	1138.58	2.36	3	1	2	111.50	
6	IIYVDDGVLSF...	156.29	2265.18	1133.60	-0.71	2	0	1	130.23	
7	FAIPAINVTS...	153.57	2159.16	1080.59	2.34	2	0	1	94.27	
8	VILFIGDGSL...	152.44	2432.33	1217.18	0.72	2	0	1	122.24	
9	TPGLSNATQV...	149.59	2121.07	1061.54	0.87	2	0	1	76.14	
10	FIAEGSNMGS...	149.52	2297.10	1149.56	2.21	2	0	1	87.82	
11	FQYIAISQSD...	149.20	1947.85	974.93	-0.59	2	0	1	54.46	Carbamidom...
12	VINDAFGIEE...	149.11	2574.30	1288.16	2.05	2	0	1	96.50	

The user selects 'Peptides Spectrum' as his user window name



Now, the user can use his new 'Peptides Spectrum' on a different Identification Summary.



List of Abbreviations

Calc. Mass : Calculated Mass

Delta MoZ : Delta Mass to Charge Ratio

Ion Parent Int. : Ion Parent Intensity

Exp. MoZ : Experimental Mass to Charge Ratio

Missed Cl. : Missed Clivage

Next AA : Next Amino-Acid

Prev. AA : Previous Amino-Acid

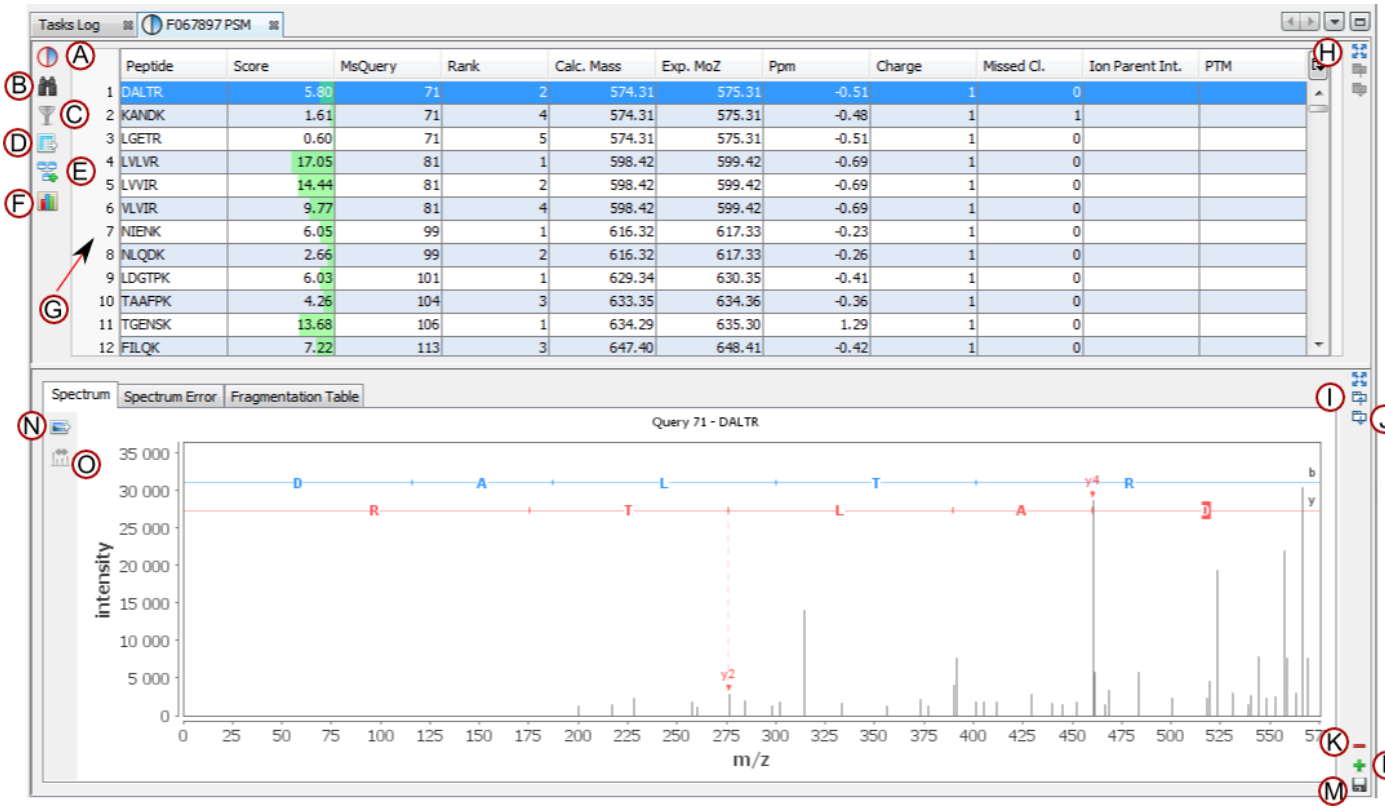
Protein S. Matches : Protein Set Matches

PSM : Peptide Spectrum Match

PTM : Post Translational Modification

RT : Retention Time

Frame Toolbars Functionnalities



A : Display Decoy Data.

B : Search in the Table. (using * and ? wild cards)

C : Filter data displayed in the Table

D : Export data displayed in the Table

E : Send to Data Mixer to compare data from different views

F : Create a Graphic : histogram or scatter plot

G : Right click on the marker bar to display Line Numbers or add Annotations/Bookmarks

H : Expands the frame to its maximum (other frames are hidden).

I : Gather the frame with the previous one as a tab.

J : Split the last tab as a frame underneath

K : Remove the last Tab or Frame

L : Open a dialog to let the user add a View (as a Frame, a Tab or a splitted Frame)

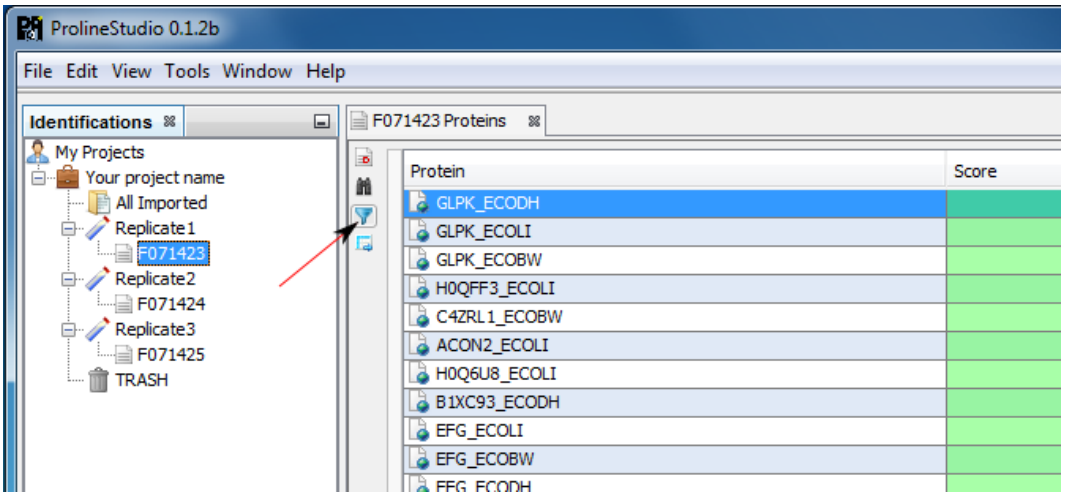
M : Save the window as a user window, to display the same window with different data later

N : Export view as an image

O : Generate Spectrum Matches

Filter Tables

You can filter data displayed in the different tables thanks to the filter button at the top right corner of a table.

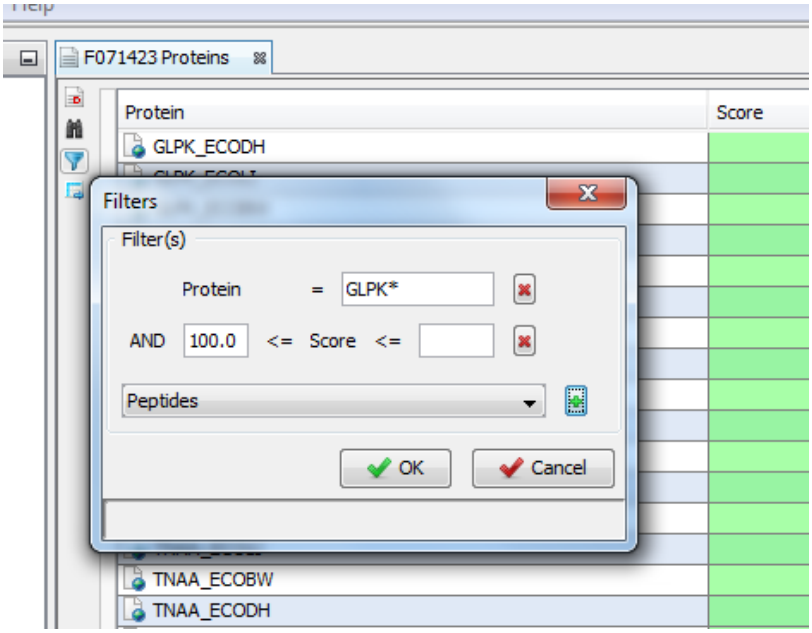


When you have clicked on the filter button, a dialog is opened. In this dialog you can select the columns of the table you want to filter thanks to the “+” button.

In the following example, we have added two filters:

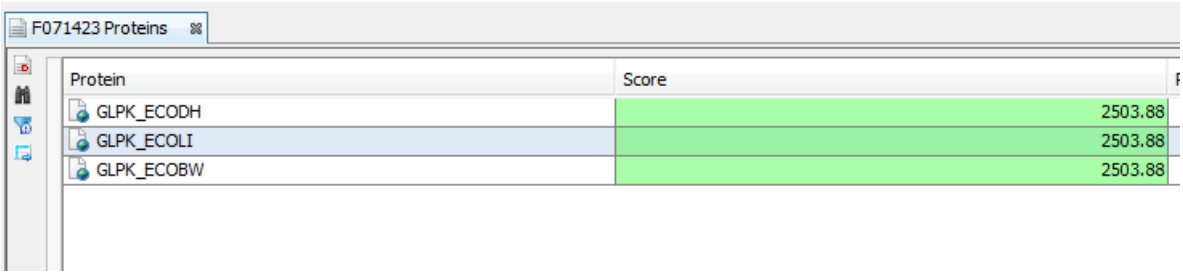
- **one on the Protein Name column** (available wildcards are * to replace multiple characters and ? to replace one character)

- **one on the Score Column** (Score must be at least 100 and there is no maximum specified).



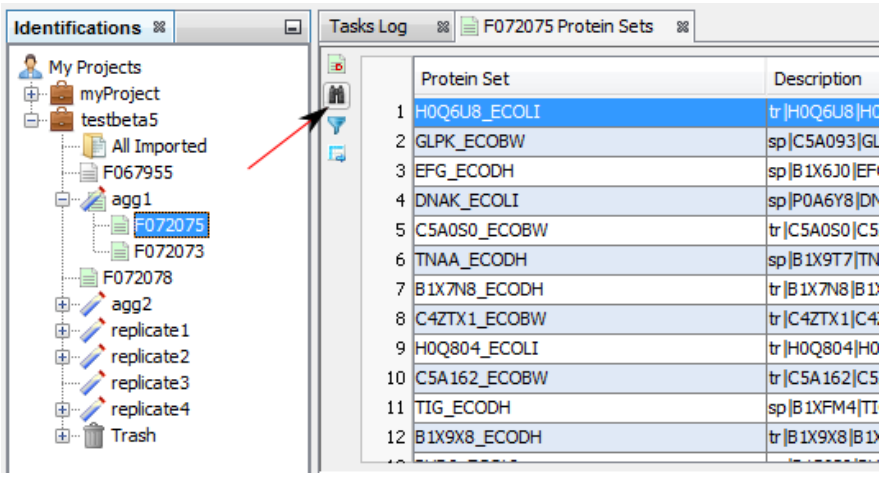
The result is all the proteins starting with GLPK (correspond to GLPK*) and with a score greater or equal than 100.

Note: for String filters, you can use the following wildcards: * matches zero or more characters, ? matches one character.



Search Tables

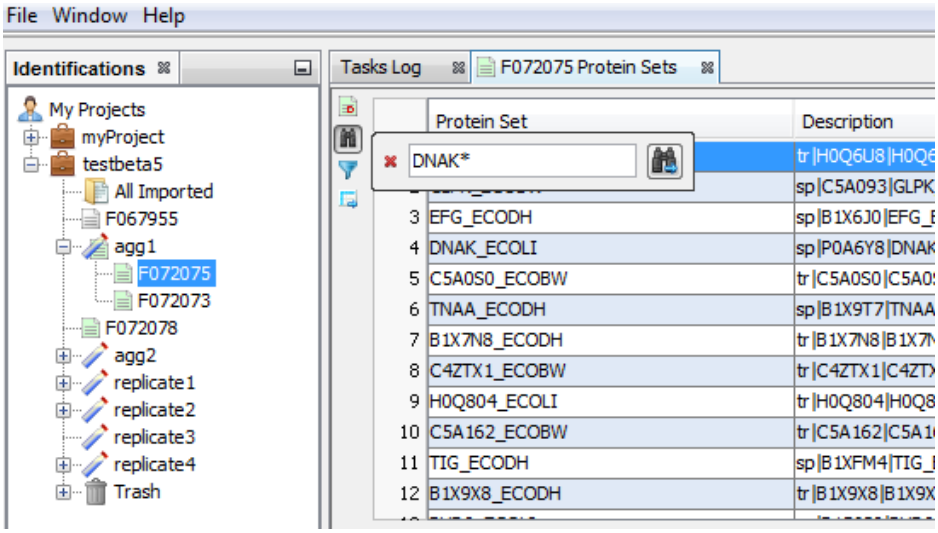
In some tables, a Search Functionality is available thanks to the search button at the top right corner.



When you have clicked on the search button, a floating panel is opened. In this panel you can fill in the searched expression. Two wild cards are available:

- '*' : can replace all characters
- '?' : can replace one character

In the following example, the user search for a ProteinSet whose name starts with "DNAK".



You can do an incremental search by clicking again on the search button of the floating panel.

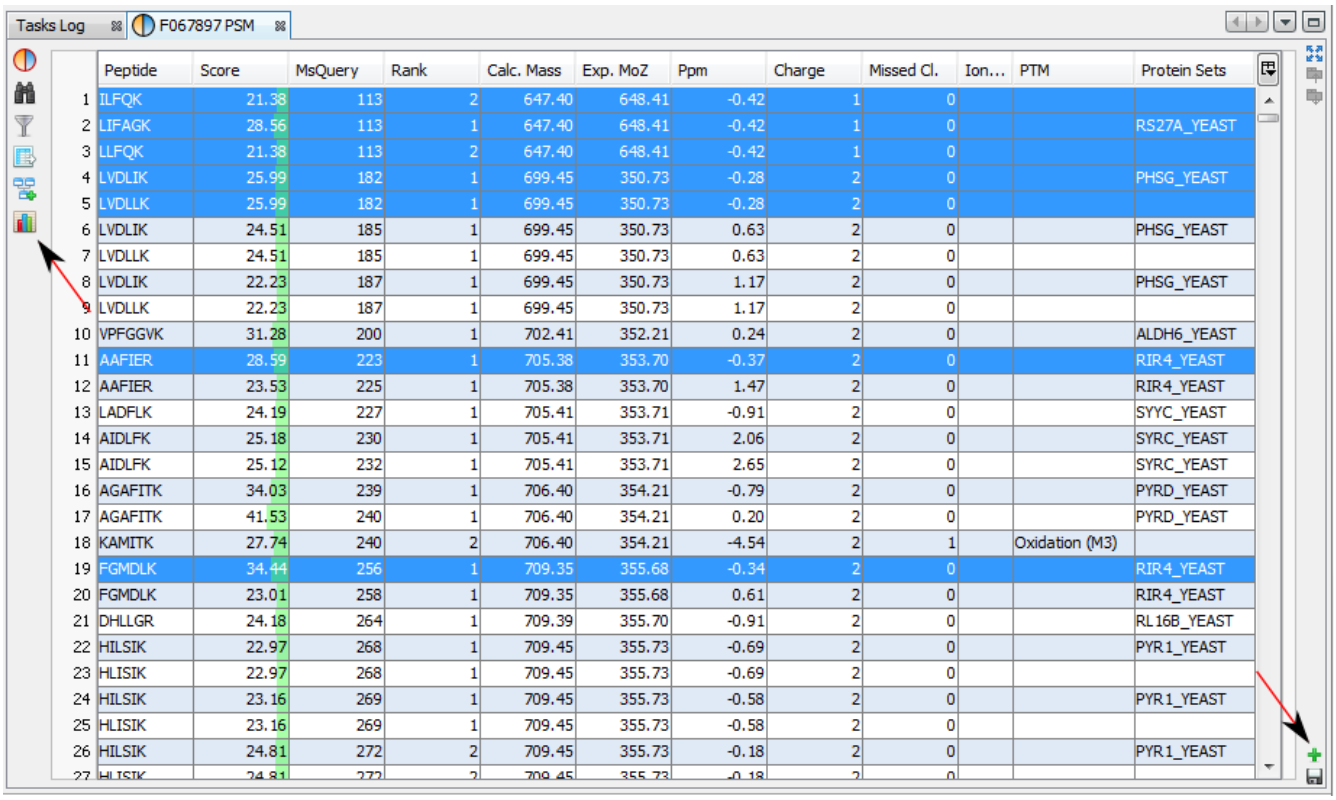
Graphics

Create a Graphic

There are two ways to obtain a graphic from data:

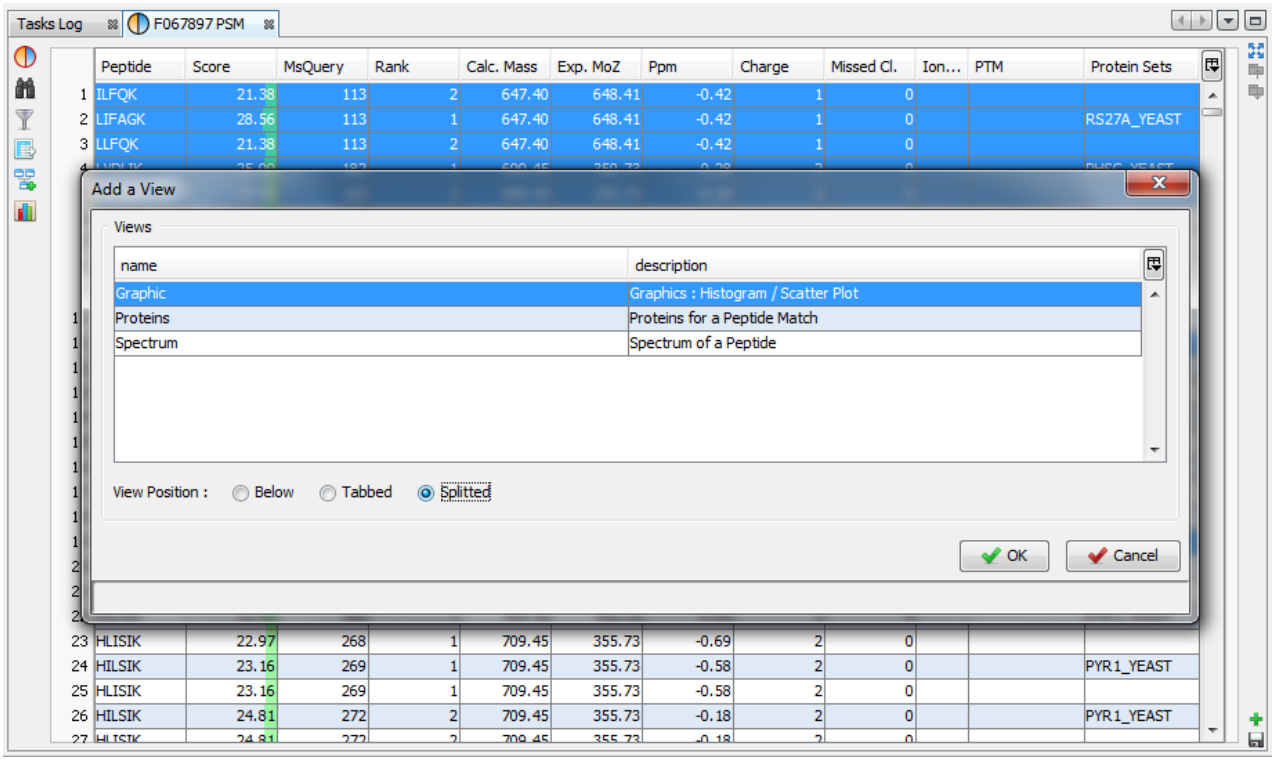
- In the windows with PSM of a Search Result or of an Identification Summary, you can ask for the display of a histogram in a new window to check the quality of your identification.

- In any window, you can click on the '+' button to add a graphic (Scatter Plot or Histogram) as a view in the same window

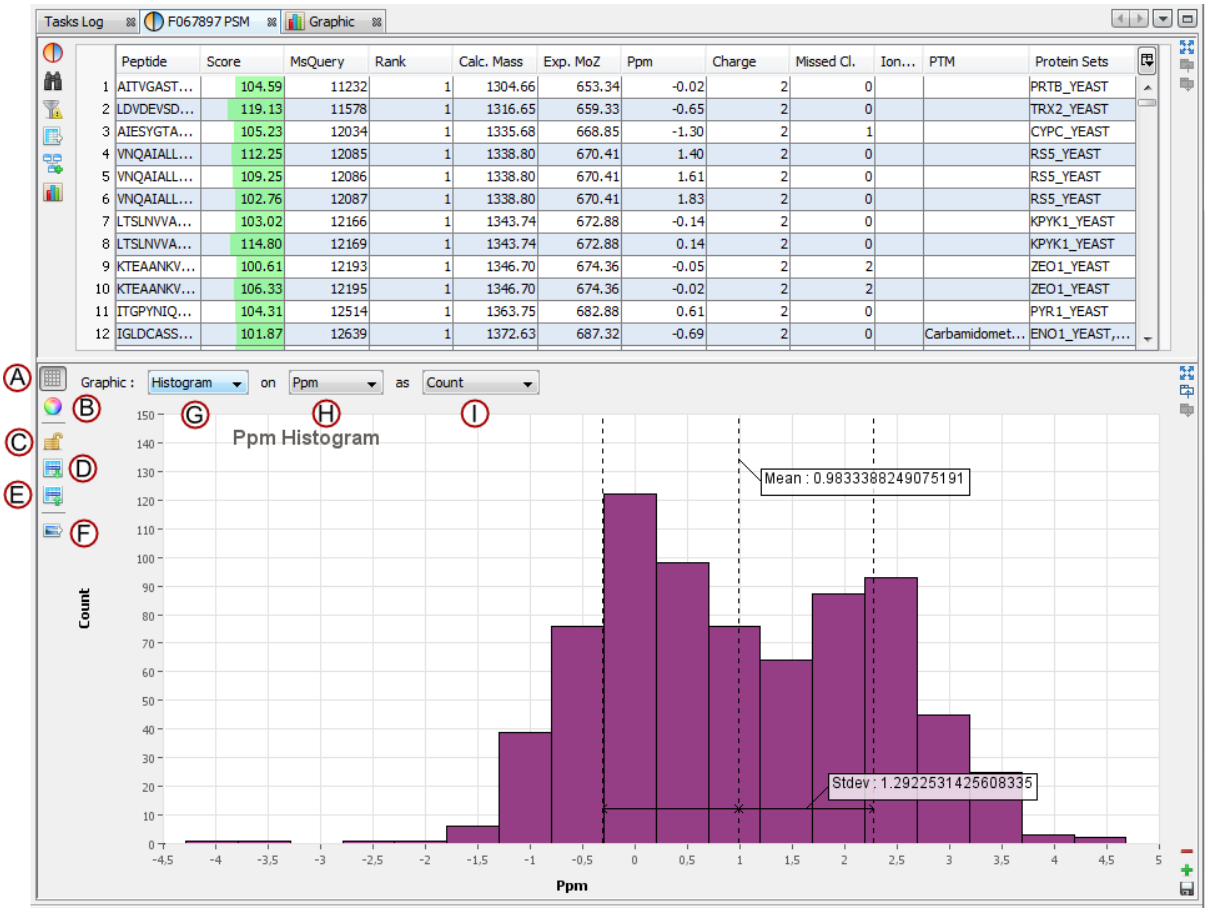


	Peptide	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion...	PTM	Protein Sets
1	ILFQK	21.38	113	2	647.40	648.41	-0.42	1	0			
2	LIFAGK	28.56	113	1	647.40	648.41	-0.42	1	0			RS27A_YEAST
3	LLFQK	21.38	113	2	647.40	648.41	-0.42	1	0			
4	LVDLIK	25.99	182	1	699.45	350.73	-0.28	2	0			PHSG_YEAST
5	LVDLLK	25.99	182	1	699.45	350.73	-0.28	2	0			
6	LVDLIK	24.51	185	1	699.45	350.73	0.63	2	0			PHSG_YEAST
7	LVDLLK	24.51	185	1	699.45	350.73	0.63	2	0			
8	LVDLIK	22.23	187	1	699.45	350.73	1.17	2	0			PHSG_YEAST
9	LVDLLK	22.23	187	1	699.45	350.73	1.17	2	0			
10	VPFGGVK	31.28	200	1	702.41	352.21	0.24	2	0			ALDH6_YEAST
11	AAFIER	28.59	223	1	705.38	353.70	-0.37	2	0			RIR4_YEAST
12	AAFIER	23.53	225	1	705.38	353.70	1.47	2	0			RIR4_YEAST
13	LADFLK	24.19	227	1	705.41	353.71	-0.91	2	0			SYYC_YEAST
14	AIDLFK	25.18	230	1	705.41	353.71	2.06	2	0			SYRC_YEAST
15	AIDLFK	25.12	232	1	705.41	353.71	2.65	2	0			SYRC_YEAST
16	AGAFITK	34.03	239	1	706.40	354.21	-0.79	2	0			PYRD_YEAST
17	AGAFITK	41.53	240	1	706.40	354.21	0.20	2	0			PYRD_YEAST
18	KAMITK	27.74	240	2	706.40	354.21	-4.54	2	1		Oxidation (M3)	
19	FGMDLK	34.44	256	1	709.35	355.68	-0.34	2	0			RIR4_YEAST
20	FGMDLK	23.01	258	1	709.35	355.68	0.61	2	0			RIR4_YEAST
21	DHLLGR	24.18	264	1	709.39	355.70	-0.91	2	0			RL16B_YEAST
22	HILSIK	22.97	268	1	709.45	355.73	-0.69	2	0			PYR1_YEAST
23	HLISIK	22.97	268	1	709.45	355.73	-0.69	2	0			
24	HILSIK	23.16	269	1	709.45	355.73	-0.58	2	0			PYR1_YEAST
25	HLISIK	23.16	269	1	709.45	355.73	-0.58	2	0			
26	HILSIK	24.81	272	2	709.45	355.73	-0.18	2	0			PYR1_YEAST
27	HLISIK	24.81	272	2	709.45	355.73	-0.18	2	0			

If you have clicked on the '+' button, the Add View Dialog is opened and you must select the Graphic View



Graphic options



A: Display/Remove Grid toggle button

B: Modify colour of the graphic

C: Lock/Unlock incoming data. If it is unlocked, the graphic is updated when the user apply a new filter to the previous view (for instance Peptide Score >= 50) If it is locked, changing filtering on the previous view does not modify the graphic.

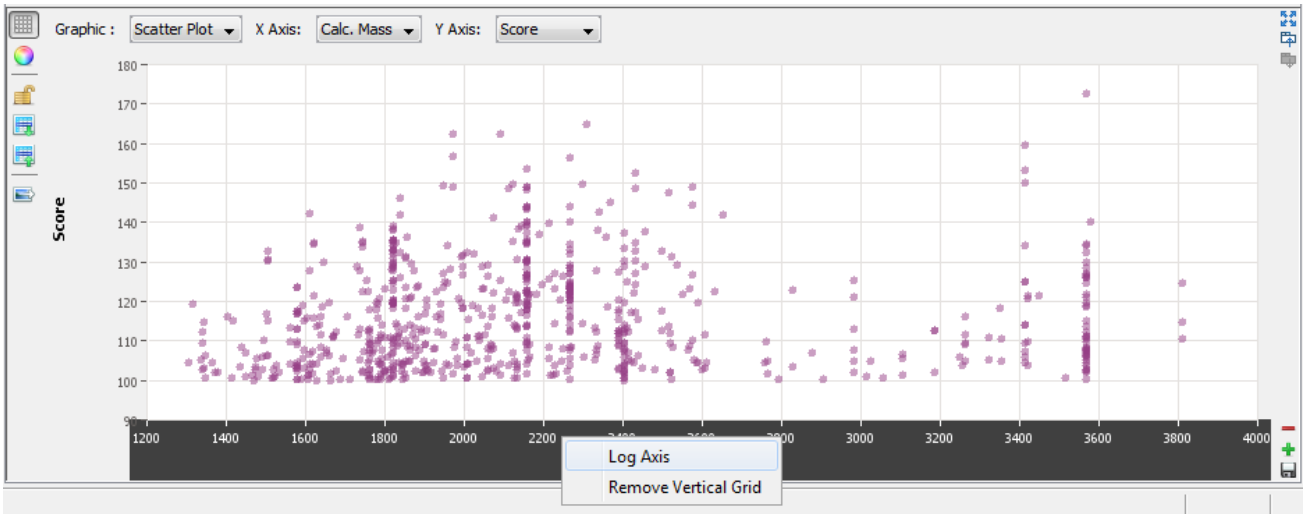
D: Select Data in the graphic according to data selected in table in the previous view.

E: Select data in the table of the previous view according to data selected in the graphic.

F: Export graphic to image

G: Select the graphic type : Scatter Plot / Histogram

H/I: Select data used for X / Y axis.



It is possible to select **linear or log axis** by right clicking on an axis.

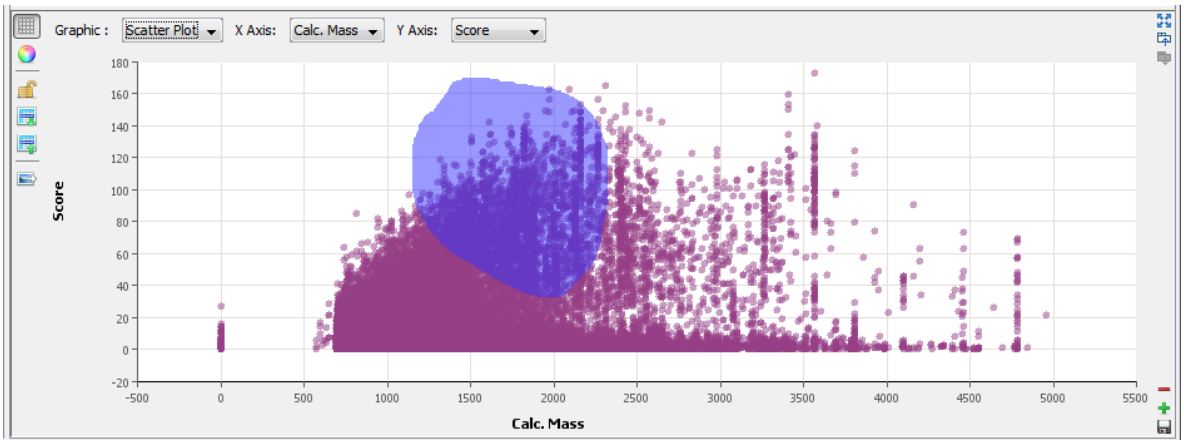
Zooming / Selection

Zoom in: Press the right mouse button and drag to the right bottom direction. A red box is displayed. Release the mouse button when you have selected the area to zoom in.

Zoom out: Press the right mouse button and drag to the left top direction. When you release the mouse button, the zooming is reset to view all.

Select: Press the left mouse button and drag the mouse to surround the data you want to select. When you release the button, the selection is done. Or left click on the data you want to select. It is possible to use Ctrl key to add to the previous selection.

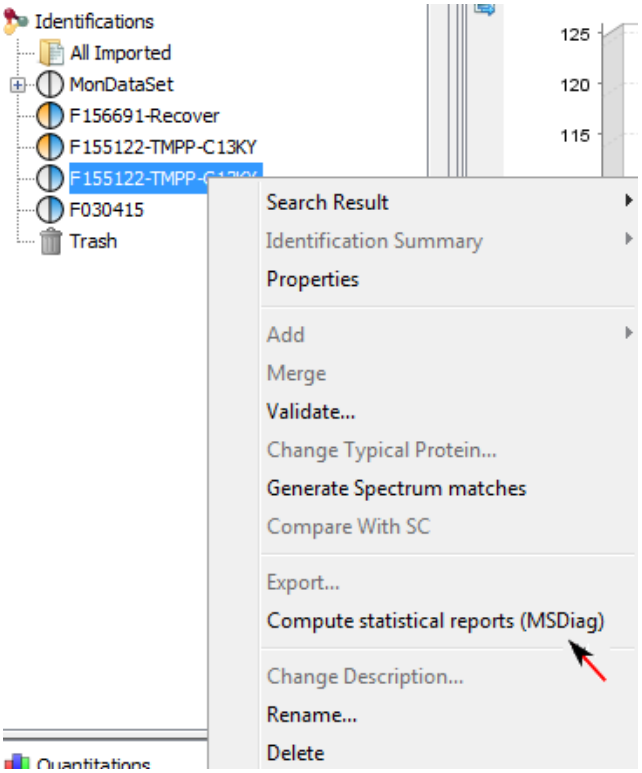
Unselect: Left click on a empty area to clear the selection.



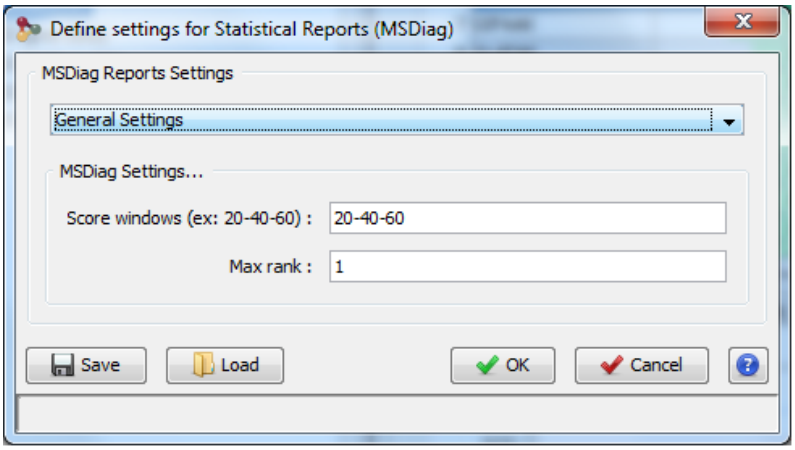
Statistical Reports (MSDiag)

In order to launch MSDiag Reports (statistical reports), simply select a node on the tree and choose 'Compute statistical reports' and wait for the results to appear. This applies to a search result only. (Not possible for a dataset)

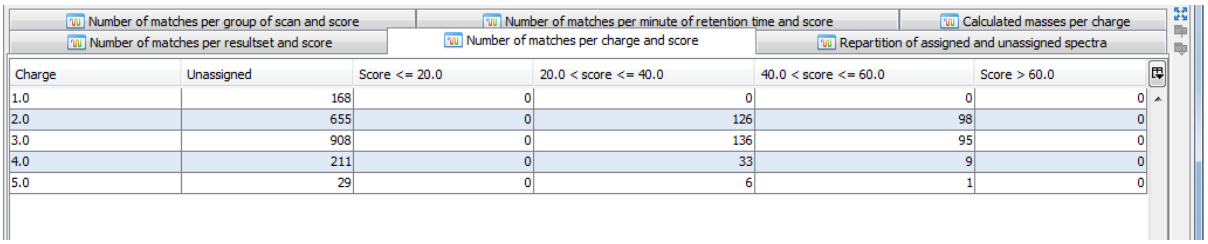
Choose the menu option:



You then configure some settings before launching the process.



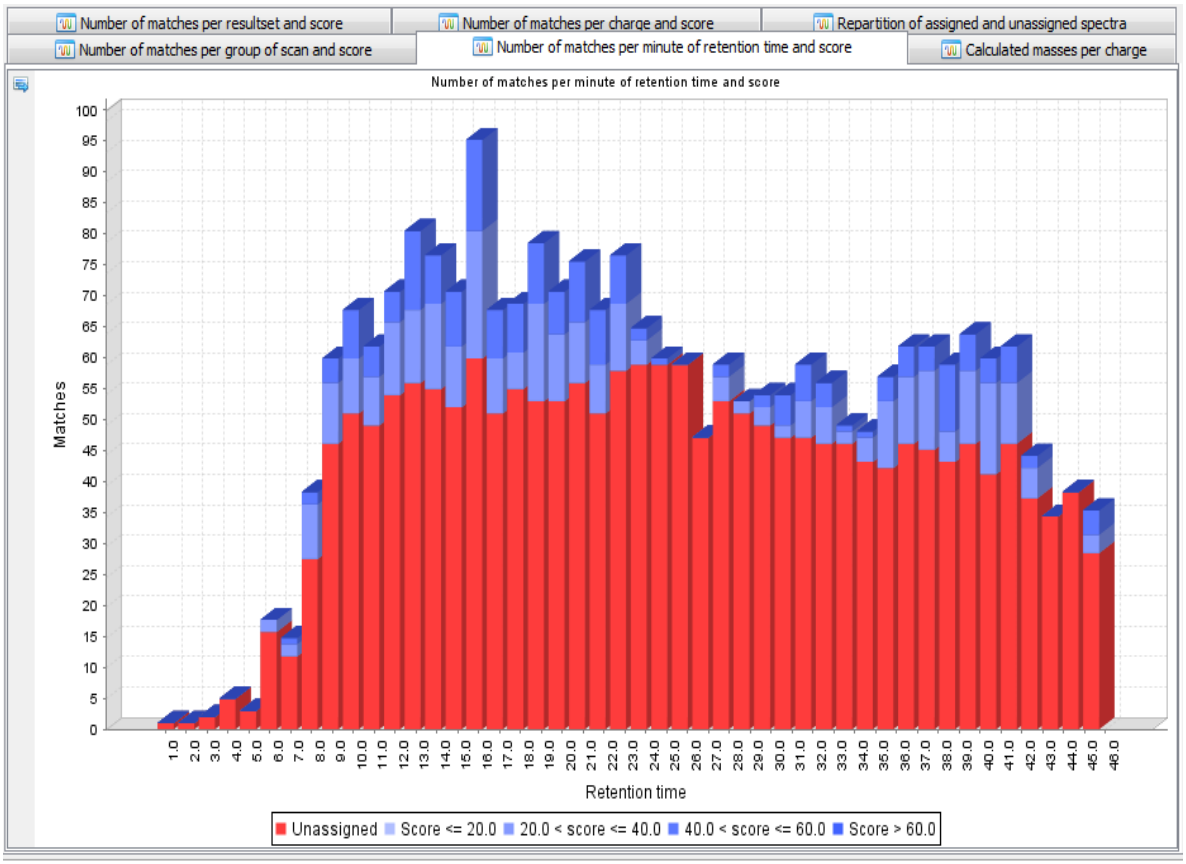
Your report will appear in a matter of seconds (depending of the amount of data to be processed).


Number of matches per group of scan and score
Number of matches per minute of retention time and score
Calculated masses per charge
Number of matches per resultset and score
Number of matches per charge and score
Repartition of assigned and unassigned spectra

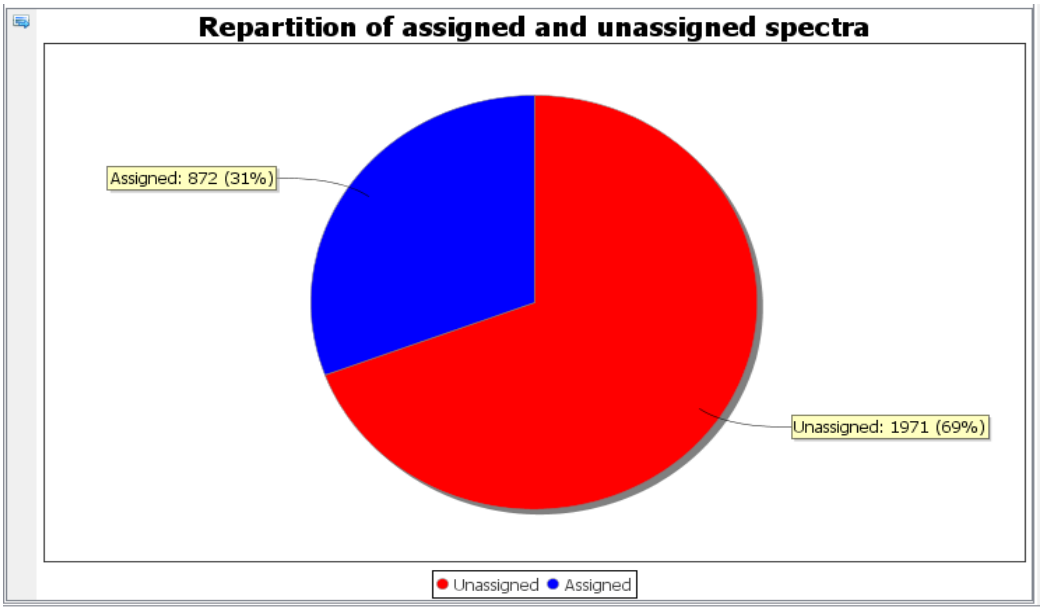
Charge	Unassigned	Score <= 20.0	20.0 < score <= 40.0	40.0 < score <= 60.0	Score > 60.0
1.0	168	0	0	0	0
2.0	655	0	126	98	0
3.0	908	0	136	95	0
4.0	211	0	33	9	0
5.0	29	0	6	1	0

You have other types of display that are possible:

Histograms:


Number of matches per resultset and score
Number of matches per charge and score
Repartition of assigned and unassigned spectra
Number of matches per group of scan and score
Number of matches per minute of retention time and score
Calculated masses per charge
Number of matches per minute of retention time and score
Matches
Retention time
Unassigned
Score <= 20.0
20.0 < score <= 40.0
40.0 < score <= 60.0
Score > 60.0

And pie charts:



Export Data / Image

There are four ways to do an export :

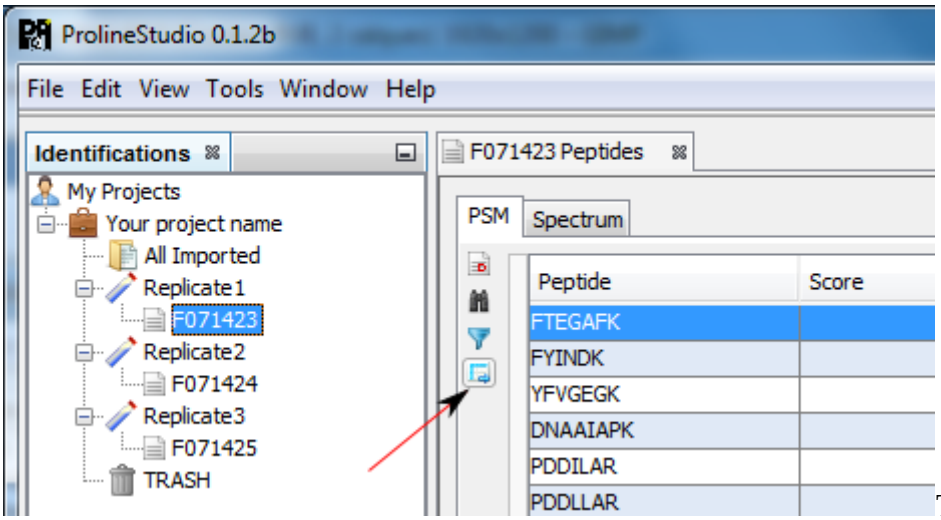
- Export a Table thanks to the export button (supported format are xlsx, xls, csv)

- Export data thanks to a Copy/Paste from the selected rows of a Table to an application like Excel.

- Export all data corresponding to an Identification Summary

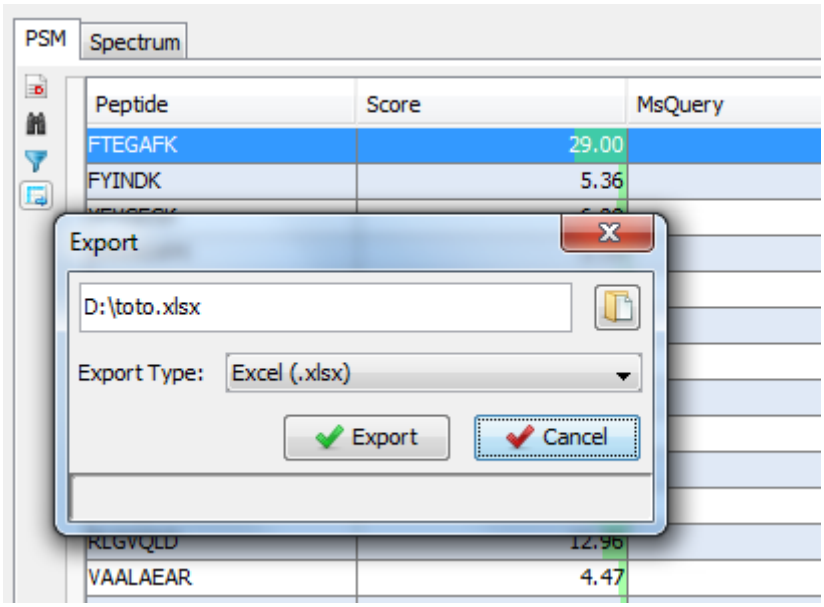
- Export an image of a view

1. Export a Table

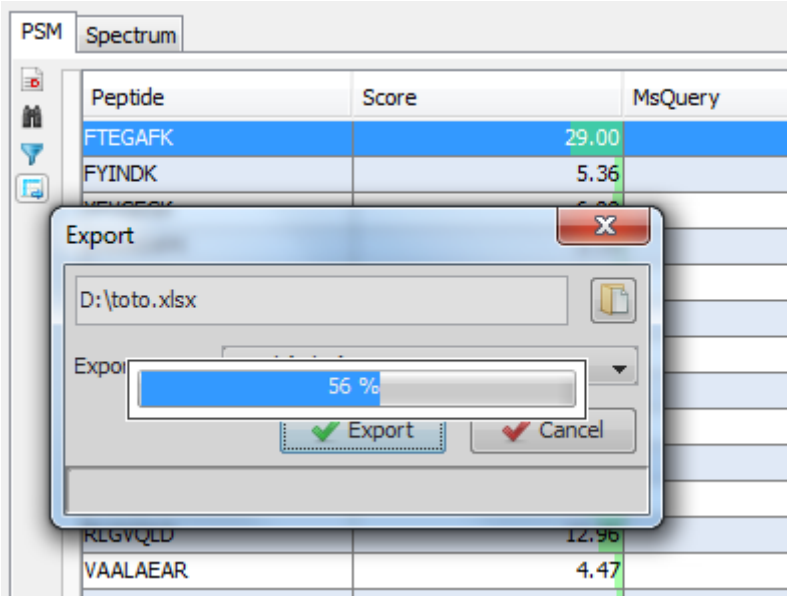


To export a table, click on the Export Button at the left top of a table.

An Export Dialog is opened, you can select the file path for the export and the type of the export (Excel (.xlsx); Excel (.xls) or CSV.)



To perform the export, click on the OK Button. The task can take a few seconds if the table has a lot of rows and so a progress bar is displayed.



2. Copy/Paste a Table

To copy/Paste a Table :

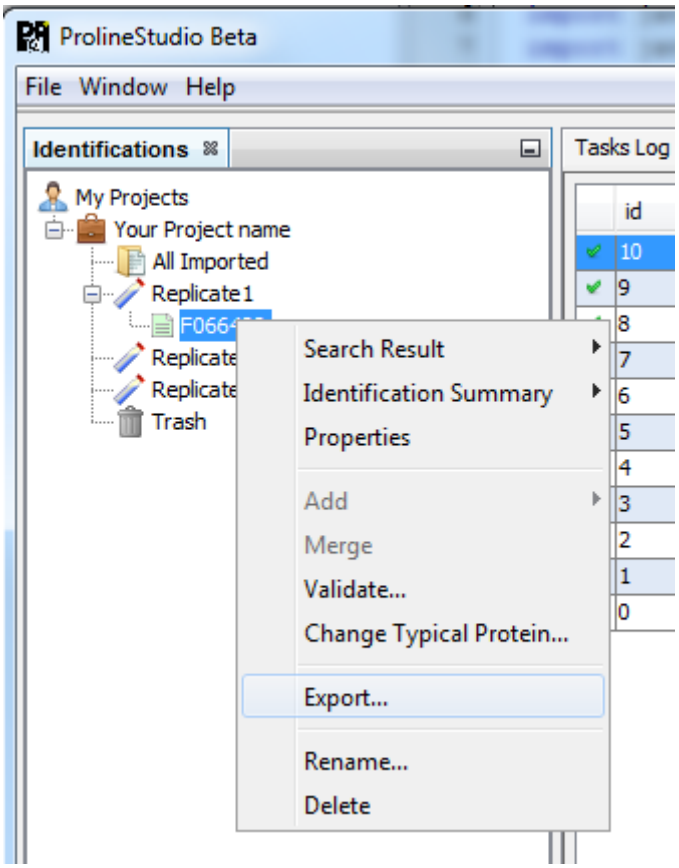
- Select rows you want to copy

- Press Ctrl and C keys in the same time

- Open for example excel and press Ctrl and V keys in the same time to paste the copied rows.

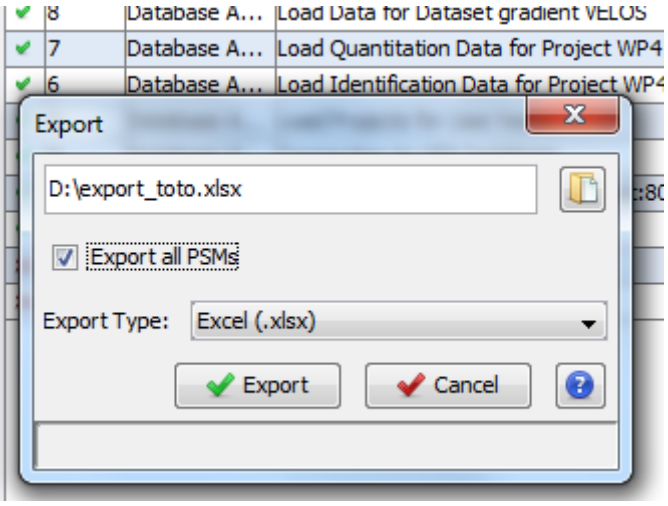
3. Export an Identification Summary

To Export all data of an Identification Summary, you must right click on a Identification Summary to open the contextual popup and select the “Export…” Menu.



An Export Dialog is opened, you can select the file path for the export and the type of the export (only Excel (.xlsx) is available for the moment).

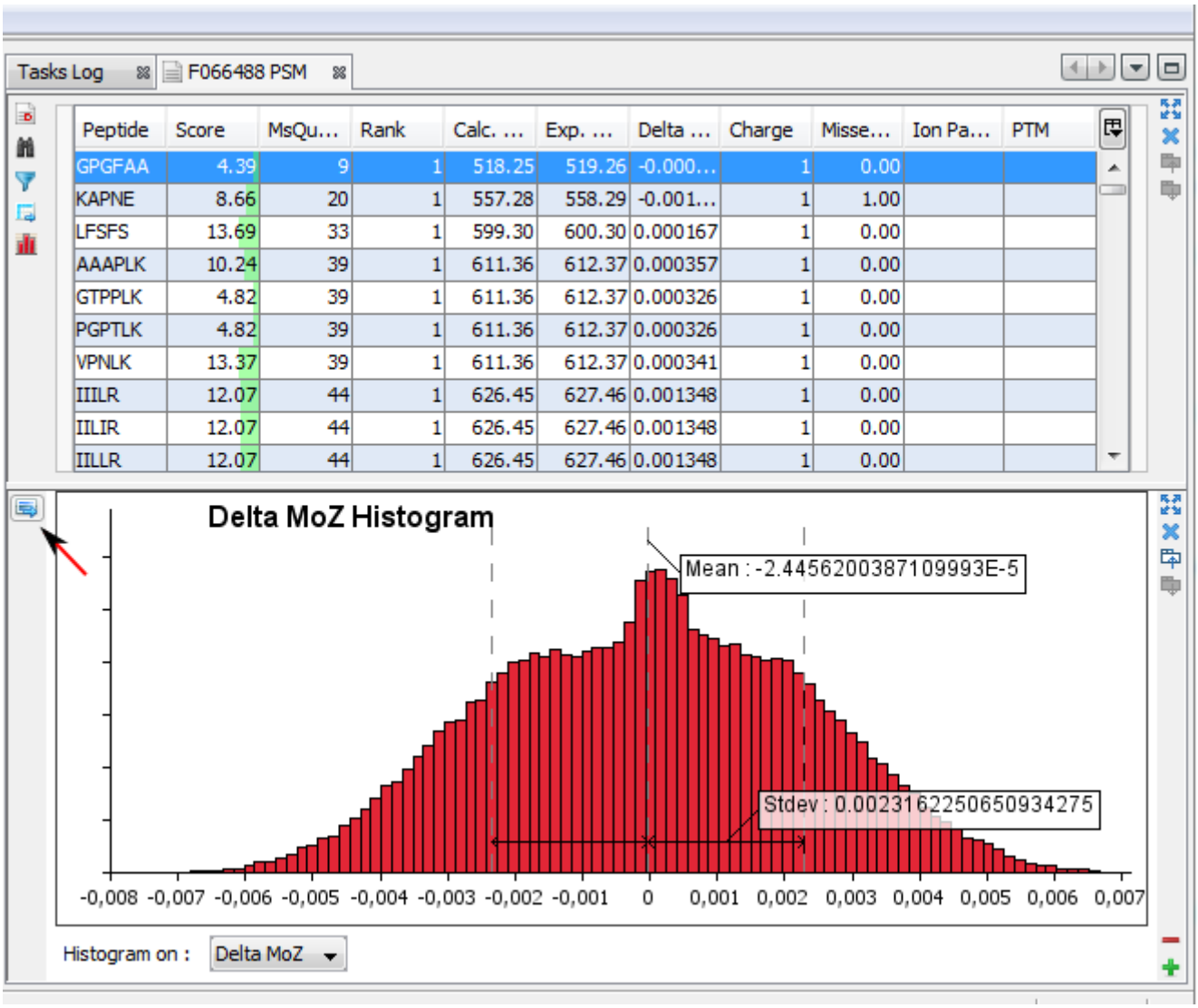
You can select the “Export All PSMs” option to add a sheet with all PSMs for each Protein Set.



Description of exported file is available here.

4. Export an Image

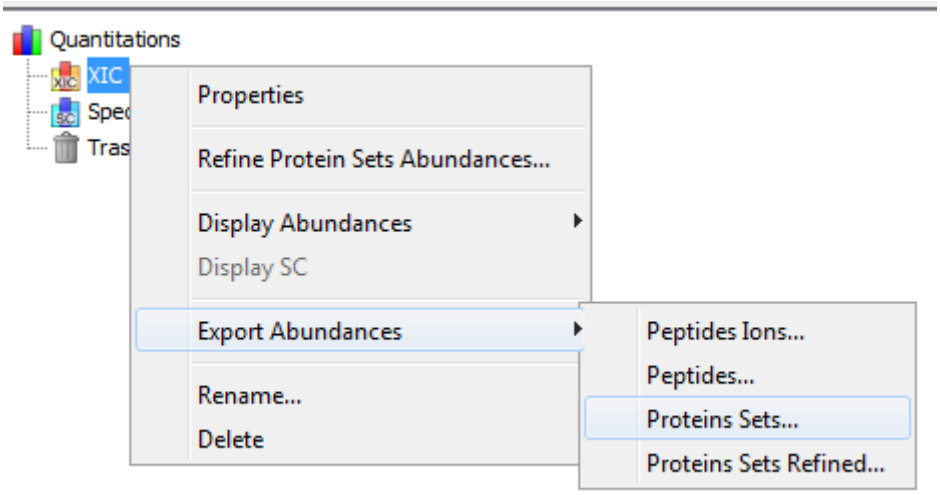
To export a graphics, click on the Export Image Button at the left top of the image.



An Export Dialog is opened, you can select the file path for the export and the type of the export

5. Export a XIC

You can export the XIC Desing values by the right mouse button popup.



You can export the abundances data at different levels:

- the protein sets

- the peptides

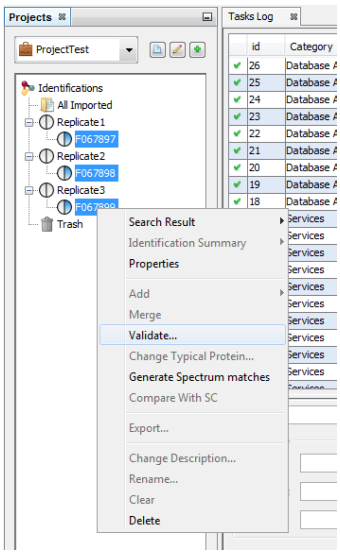
- the peptides ions

- the refined protein sets abundances (see Refine Protein Sets Abundances)

How to validate a Search Result

See description of Validation Algorithm.

Starting Validation



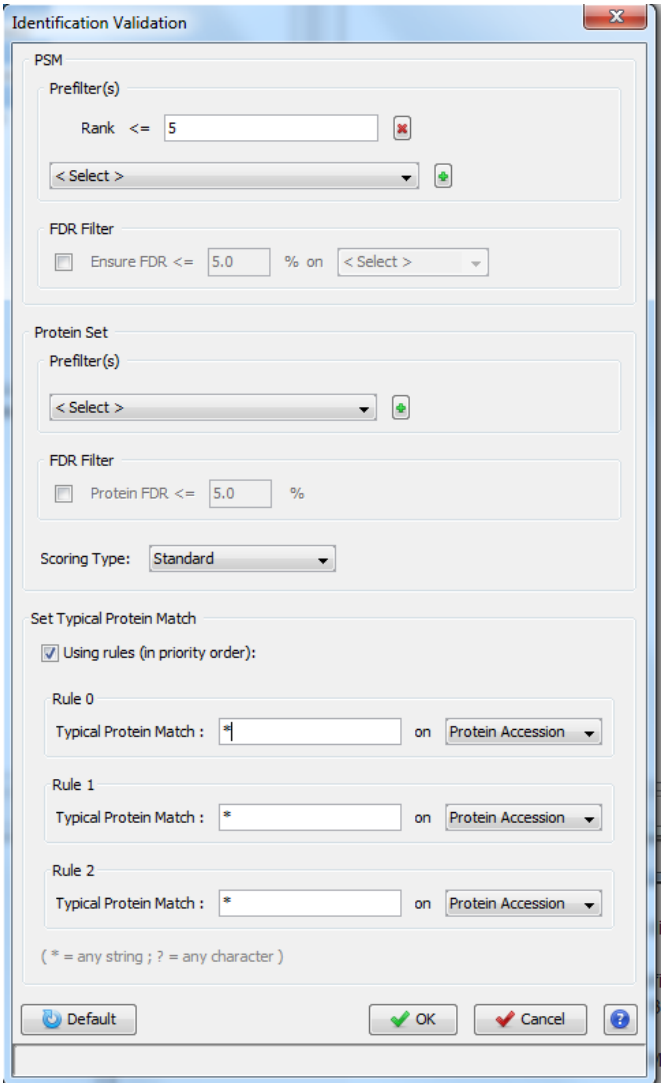
To validate a Search Result:

- Select one or multiple Search Results to validate

- Right Click to display the popup

- Click on “Validate…” menu

Validation Dialog



In the Validation Dialog, fill the different Parameters (see Validation description) :

- you can add multiple PSM Prefilter Parameters (Rank, Length, Score, e-Value, Identity p-Value, Homology p-Value) by selecting them in the combobox and clicking on Add Button '+'

- you can ensure a FDR on PSM which will reached according to the variable selected (Score, e-Value, Identity p-Value, Homology p-Value,…)

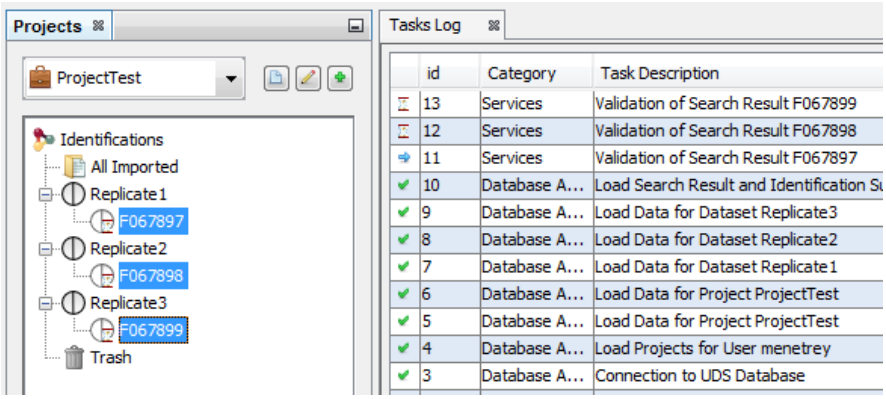
- you can add a Protein Set Prefilter on Specific Peptides.

- you can ensure a FDR on protein Sets.

- you can set the choice for the Typical Protein of a Protein Set by using a match string with wildcards (* or ?) on Protein Accession or Protein Description. (See Chang Typical Protein of Protein Sets)

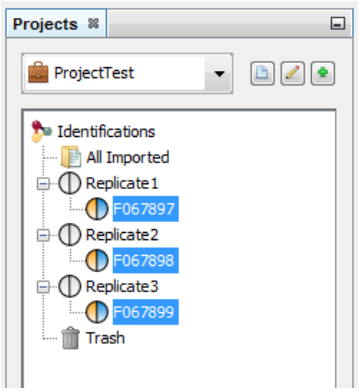
Note: FDR can be used only for Search Results with Decoy Data.

Validation Processing


Projects
ProjectTest
Identifications
All Imported
Replicate1
F067897
Replicate2
F067898
Replicate3
F067899
Trash
Tasks Log
id
Category
Task Description
13 Services Validation of Search Result F067899
12 Services Validation of Search Result F067898
11 Services Validation of Search Result F067897
10 Database A... Load Search Result and Identification S
9 Database A... Load Data for Dataset Replicate3
8 Database A... Load Data for Dataset Replicate2
7 Database A... Load Data for Dataset Replicate1
6 Database A... Load Data for Project ProjectTest
5 Database A... Load Data for Project ProjectTest
4 Database A... Load Projects for User menetrey
3 Database A... Connection to UDS Database

Validating a Search Result can take some time. While it is not finished, the Search Results are shown greyed with an hour glass over them. The tasks are displayed as running in the "Tasks Log Dialog".

Validation Done

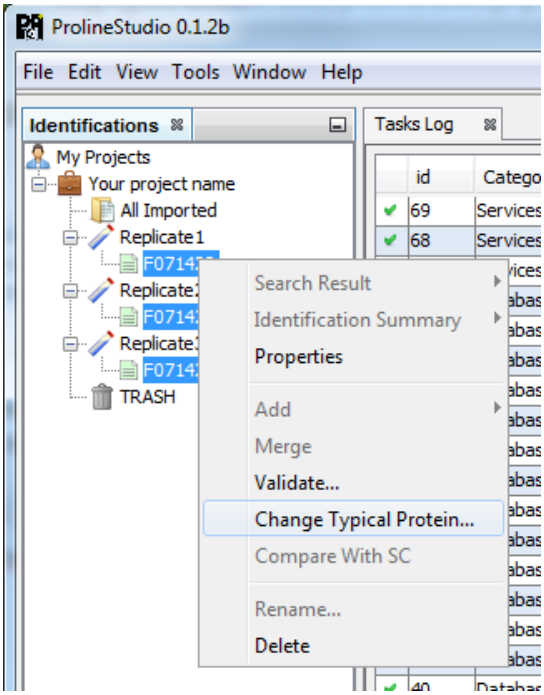


When the validation is finished, the icon becomes orange and blue. Orange part corresponds to the Identification Summary. Blue is for the Search Result part.

Change Typical Protein of Protein Sets

The protein sets windows are not updates after a Change Typical Protein ! You should close and reopen the window

Open the Dialog



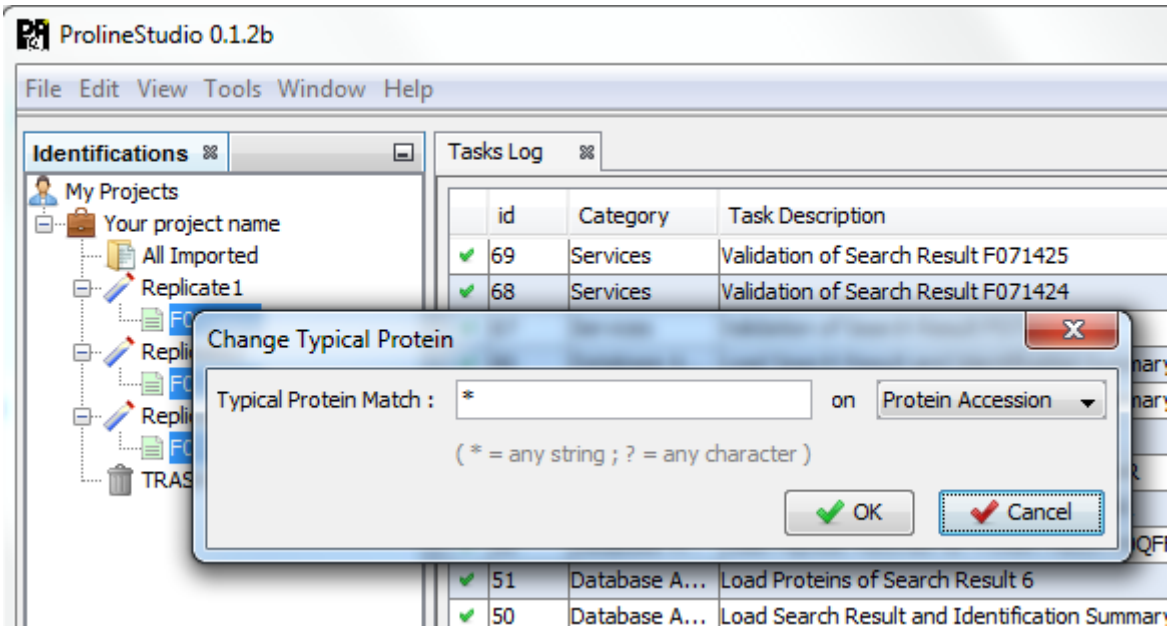
To change the Typical Protein of the Protein Sets of a Identification Summary:

- Select one or multiple Identification Summaries

- Right Click to display the popup

- Click on “Change Typical Protein…” menu

Dialog Parameters



You can set the choice for the Typical Protein of Protein Sets by using a match string with wildcards (* or ?) on Protein Accession or Protein Description.

Three rules could be specified and they will be applied in priority order. In a Protein Set, if no proteins satisfy the first rule, the second one will ne tested and so on.

Processing

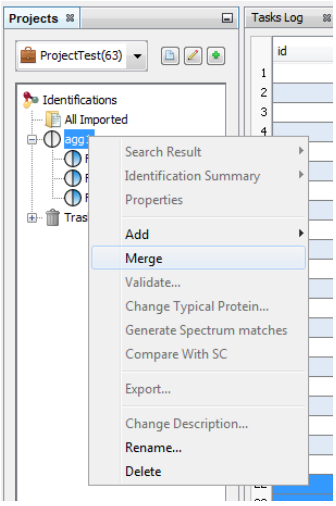
The modification of Typical Proteins can take some time. During the processing, Identification Summaries are displayed grayed with an hourglass and the tasks are displayed in the Tasks Log Window

---

Merge

Merge can be done on Search Results or on Identification Summaries. See description for Search Results merging and Identification Summaries merging

Merge on Search Results



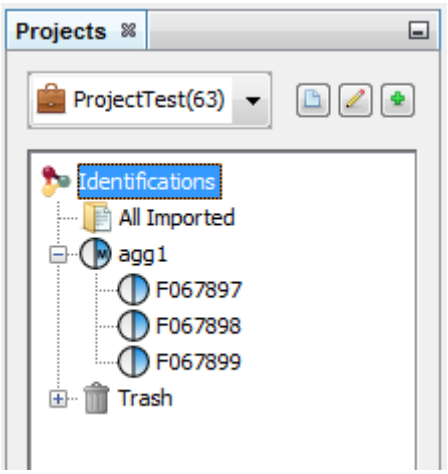
To merge a dataset with multiple Search Results:

- Select the parent dataset

- Right Click to display the popup

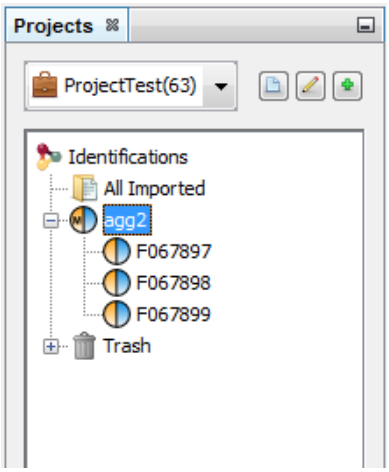
- Click on “Merge” menu

When the merge is finished, the dataset is displayed with an M in the blue part of the icon, indicating that the Merge has been done at a Search Result level.



Merge on Identification Summaries

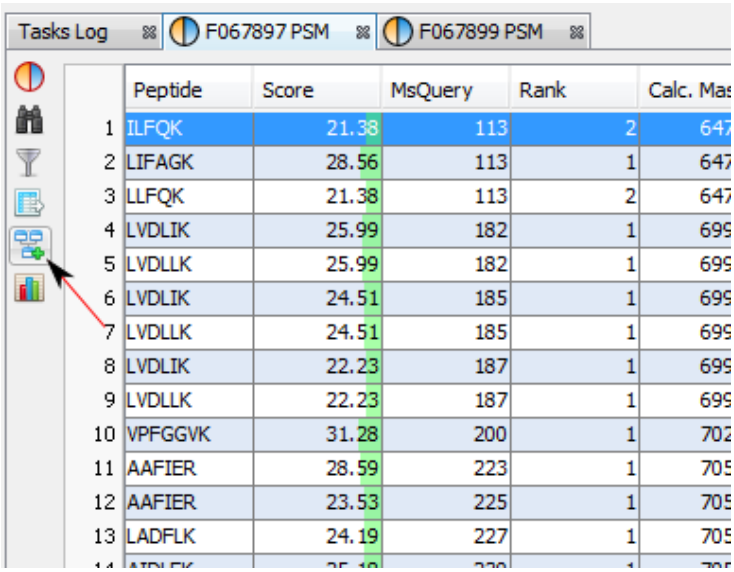
If you merge a dataset containing Identifications Summaries. The merge is done on a Identification Summary level. Therefore the dataset is displayed with an M in the orange part of the icon.



Data Mixer

The purpose of a Data Mixer is to compare / join data from different tables.

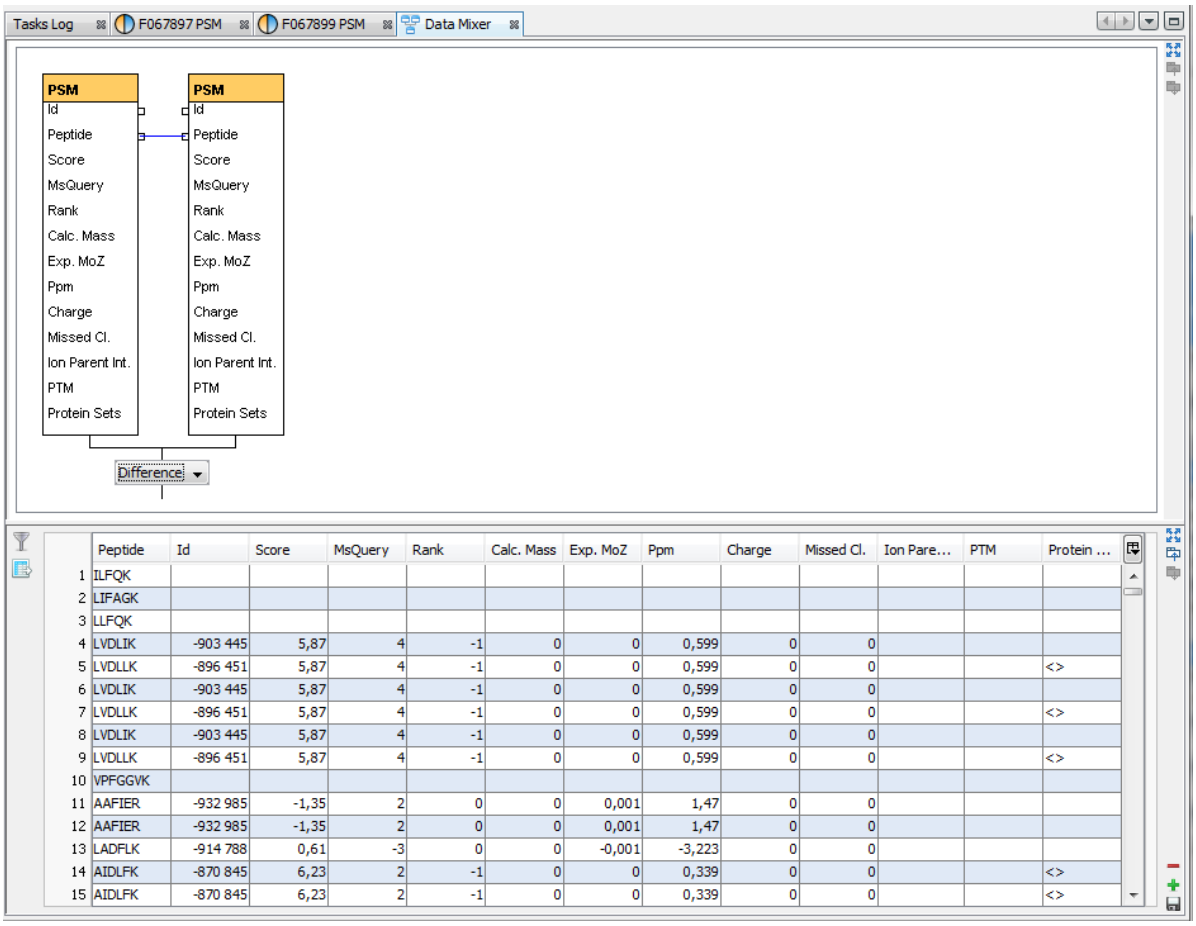
To send data to the data mixer, you can use the dedicated button that you can find in the toolbar of all views.



When you have sent data from two different views (in the following example from the PSM view of two different identification summaries). You obtain a new window with the two tables linked and you can apply a difference algorithm or a join algorithm.

Difference Algorithm

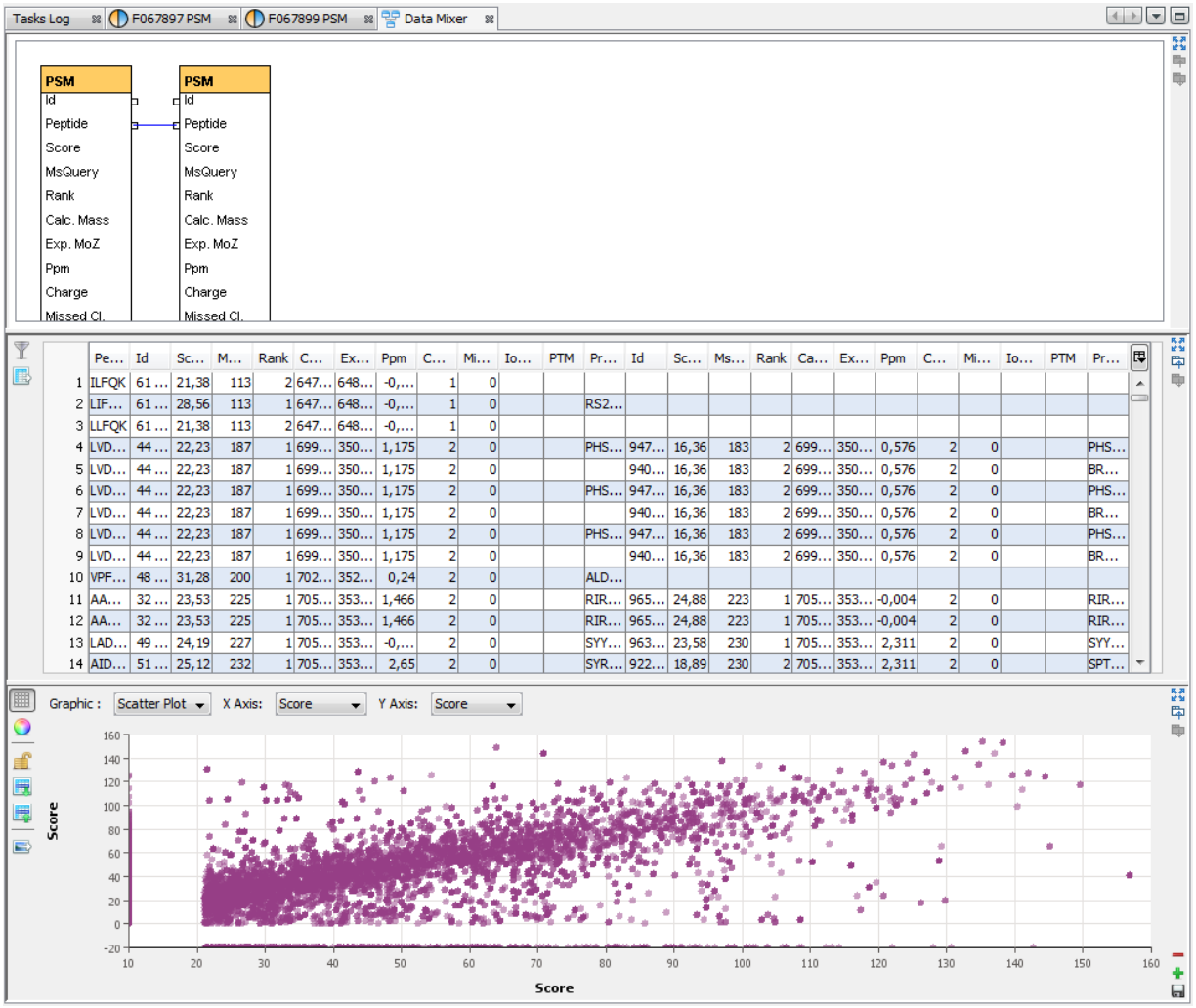
For the difference algorithm: when a key value is not found in one of the data source table, the line is displayed as empty. For numerical values a difference is done and for string values, the '<>' symbol is displayed when values are different.



	Peptide	Id	Score	MsQuery	Rank	Calc. Mass	Exp. MoZ	Ppm	Charge	Missed Cl.	Ion Pare...	PTM	Protein ...
1	ILFQK												
2	LIFAGK												
3	LLFQK												
4	LVDLIK	-903 445	5,87	4	-1	0	0	0,599	0	0			
5	LVDLLK	-896 451	5,87	4	-1	0	0	0,599	0	0			<>
6	LVDLIK	-903 445	5,87	4	-1	0	0	0,599	0	0			
7	LVDLLK	-896 451	5,87	4	-1	0	0	0,599	0	0			<>
8	LVDLIK	-903 445	5,87	4	-1	0	0	0,599	0	0			
9	LVDLLK	-896 451	5,87	4	-1	0	0	0,599	0	0			<>
10	VPFGGVK												
11	AAFIER	-932 985	-1,35	2	0	0	0,001	1,47	0	0			
12	AAFIER	-932 985	-1,35	2	0	0	0,001	1,47	0	0			
13	LADFLK	-914 788	0,61	-3	0	0	-0,001	-3,223	0	0			
14	AIDLFK	-870 845	6,23	2	-1	0	0	0,339	0	0			<>
15	AIDLFK	-870 845	6,23	2	-1	0	0	0,339	0	0			<>

Join Algorithm

In the following example, we have used the join algorithm and added a graphics thanks to the '+' to compare the scores of the PSM from two identification summaries.

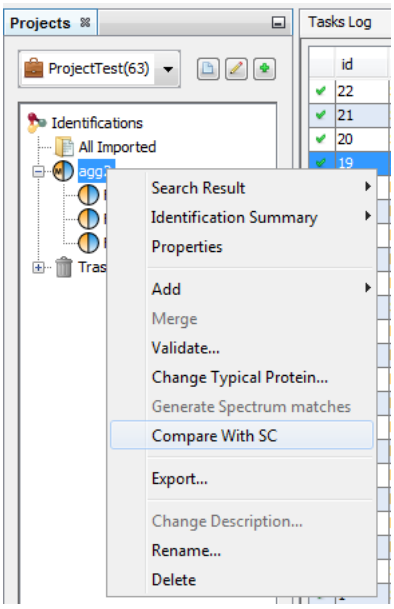


Spectral Count

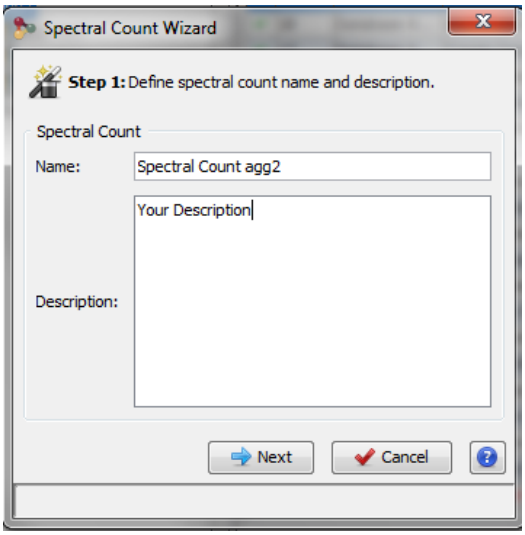
See description of [Compare Identification Summaries with Spectral Count](#) .

Generate a Spectral Count

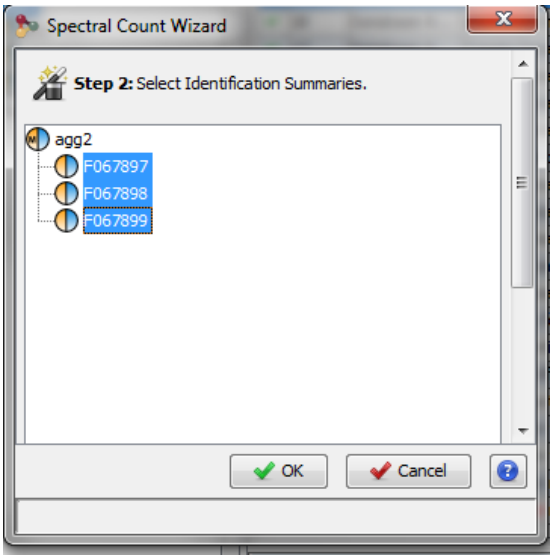
To obtain a spectral count, right click on a Dataset with merged Identification Summaries and select the “Compare with SC” menu in the popup.



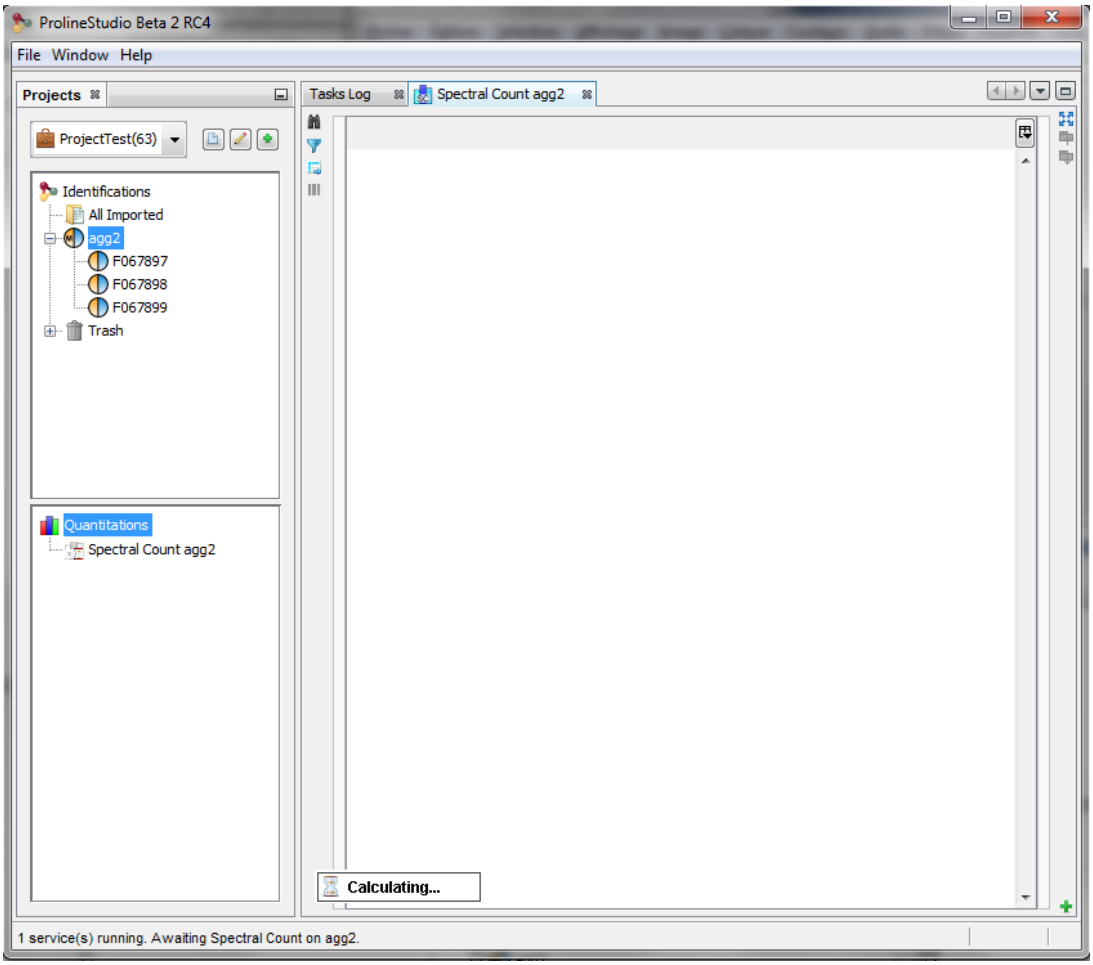
In the Spectral Count window, fill the name and description of your Spectral Count and press Next.



Then select the Identification Summaries on which you want to perform the Spectral Count and press OK.

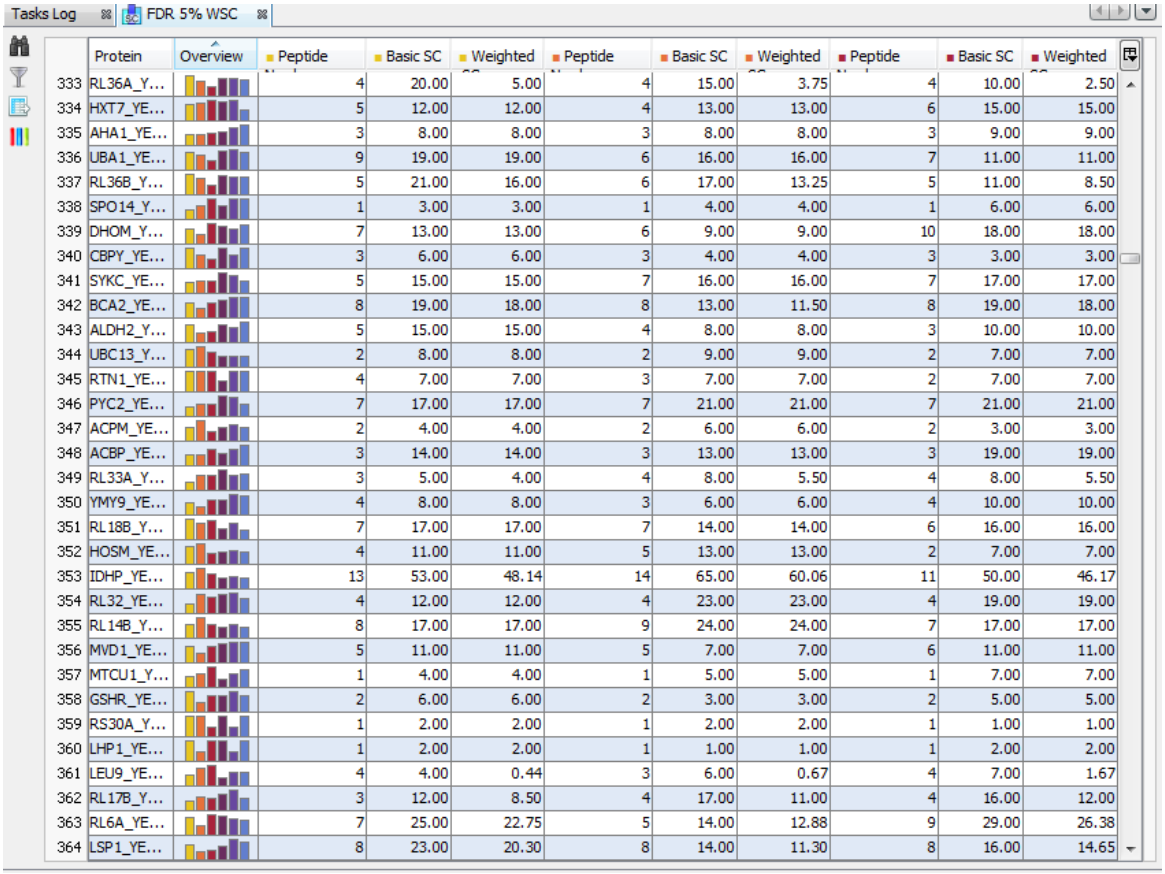


A Spectral Count window is opened with a label indicating that the calculation is being done, and the Spectral Count is added to the Quantitation's Panel.



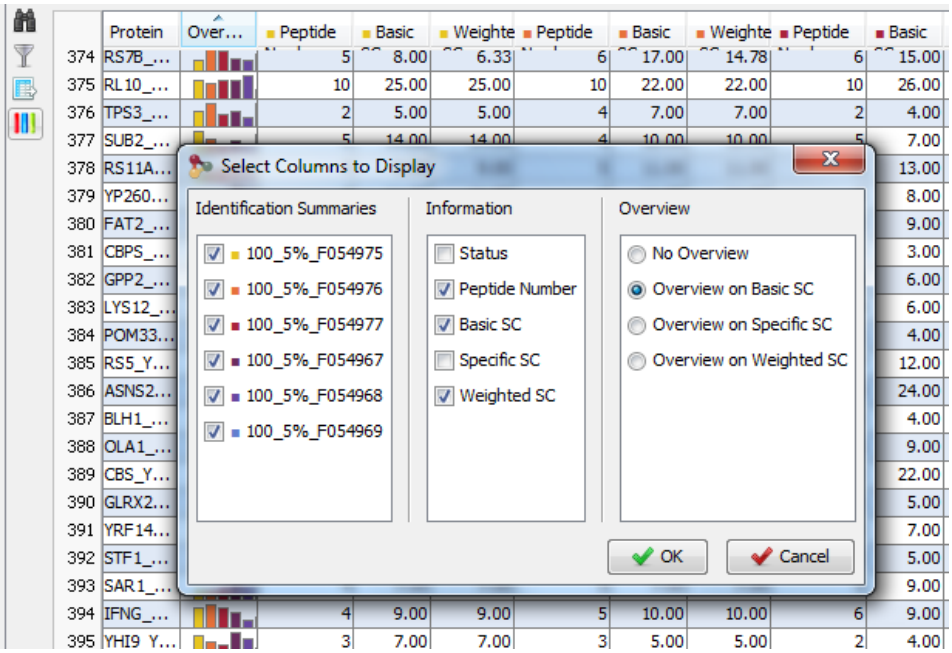
Spectral Count Result

In the Spectral Count Result Table, you will find three types of Spectral Count: Basic, Specific and Weighted, and an overview column to rapidly compare the Spectral Count between the different Identification Summaries.



	Protein	Overview	Peptide	Basic SC	Weighted	Peptide	Basic SC	Weighted	Peptide	Basic SC	Weighted
333	RL36A_Y...		4	20.00	5.00	4	15.00	3.75	4	10.00	2.50
334	HXT7_YE...		5	12.00	12.00	4	13.00	13.00	6	15.00	15.00
335	AHA1_YE...		3	8.00	8.00	3	8.00	8.00	3	9.00	9.00
336	UBA1_YE...		9	19.00	19.00	6	16.00	16.00	7	11.00	11.00
337	RL36B_Y...		5	21.00	16.00	6	17.00	13.25	5	11.00	8.50
338	SPO14_Y...		1	3.00	3.00	1	4.00	4.00	1	6.00	6.00
339	DHOM_Y...		7	13.00	13.00	6	9.00	9.00	10	18.00	18.00
340	CBPY_YE...		3	6.00	6.00	3	4.00	4.00	3	3.00	3.00
341	SYKC_YE...		5	15.00	15.00	7	16.00	16.00	7	17.00	17.00
342	BCA2_YE...		8	19.00	18.00	8	13.00	11.50	8	19.00	18.00
343	ALDH2_Y...		5	15.00	15.00	4	8.00	8.00	3	10.00	10.00
344	UBC13_Y...		2	8.00	8.00	2	9.00	9.00	2	7.00	7.00
345	RTN1_YE...		4	7.00	7.00	3	7.00	7.00	2	7.00	7.00
346	PYC2_YE...		7	17.00	17.00	7	21.00	21.00	7	21.00	21.00
347	ACPM_YE...		2	4.00	4.00	2	6.00	6.00	2	3.00	3.00
348	ACBP_YE...		3	14.00	14.00	3	13.00	13.00	3	19.00	19.00
349	RL33A_Y...		3	5.00	4.00	4	8.00	5.50	4	8.00	5.50
350	YMY9_YE...		4	8.00	8.00	3	6.00	6.00	4	10.00	10.00
351	RL18B_Y...		7	17.00	17.00	7	14.00	14.00	6	16.00	16.00
352	HOSM_YE...		4	11.00	11.00	5	13.00	13.00	2	7.00	7.00
353	IDHP_YE...		13	53.00	48.14	14	65.00	60.06	11	50.00	46.17
354	RL32_YE...		4	12.00	12.00	4	23.00	23.00	4	19.00	19.00
355	RL14B_Y...		8	17.00	17.00	9	24.00	24.00	7	17.00	17.00
356	MVD1_YE...		5	11.00	11.00	5	7.00	7.00	6	11.00	11.00
357	MTCU1_Y...		1	4.00	4.00	1	5.00	5.00	1	7.00	7.00
358	GSHR_YE...		2	6.00	6.00	2	3.00	3.00	2	5.00	5.00
359	RS30A_Y...		1	2.00	2.00	1	2.00	2.00	1	1.00	1.00
360	LHP1_YE...		1	2.00	2.00	1	1.00	1.00	1	2.00	2.00
361	LEU9_YE...		4	4.00	0.44	3	6.00	0.67	4	7.00	1.67
362	RL17B_Y...		3	12.00	8.50	4	17.00	11.00	4	16.00	12.00
363	RL6A_YE...		7	25.00	22.75	5	14.00	12.88	9	29.00	26.38
364	LSP1_YE...		8	23.00	20.30	8	14.00	11.30	8	16.00	14.65

The overview is based on the Basic Spectral Count, but it can be changed thanks to the Column Buttons. This button allows changing the visibility of the columns too.



Comparing Spectral Counts

If you sort the column Overview by clicking on its header, you will be able to easily find the proteins with Spectral Counts different from one Identification Summary to another.

Display a Spectral Count

You can later display again an already generated Spectral Count, see Display a Spectral Count

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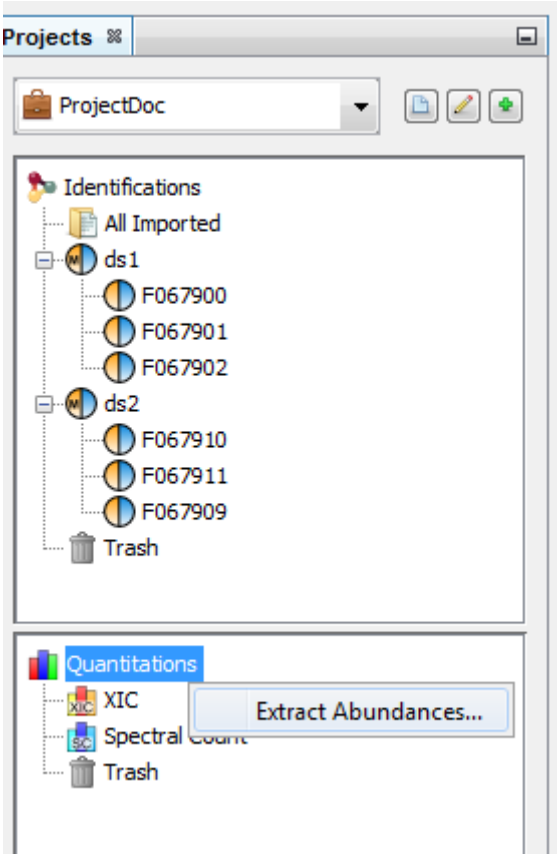
XIC Quantitation

For description on LCMS Quantitation you can first read the principles in this page: Quantitation : principles

Generate a XIC

To generate a XIC, right click on the Quantitations Node and select the “Extract Abundances” menu in the popup.

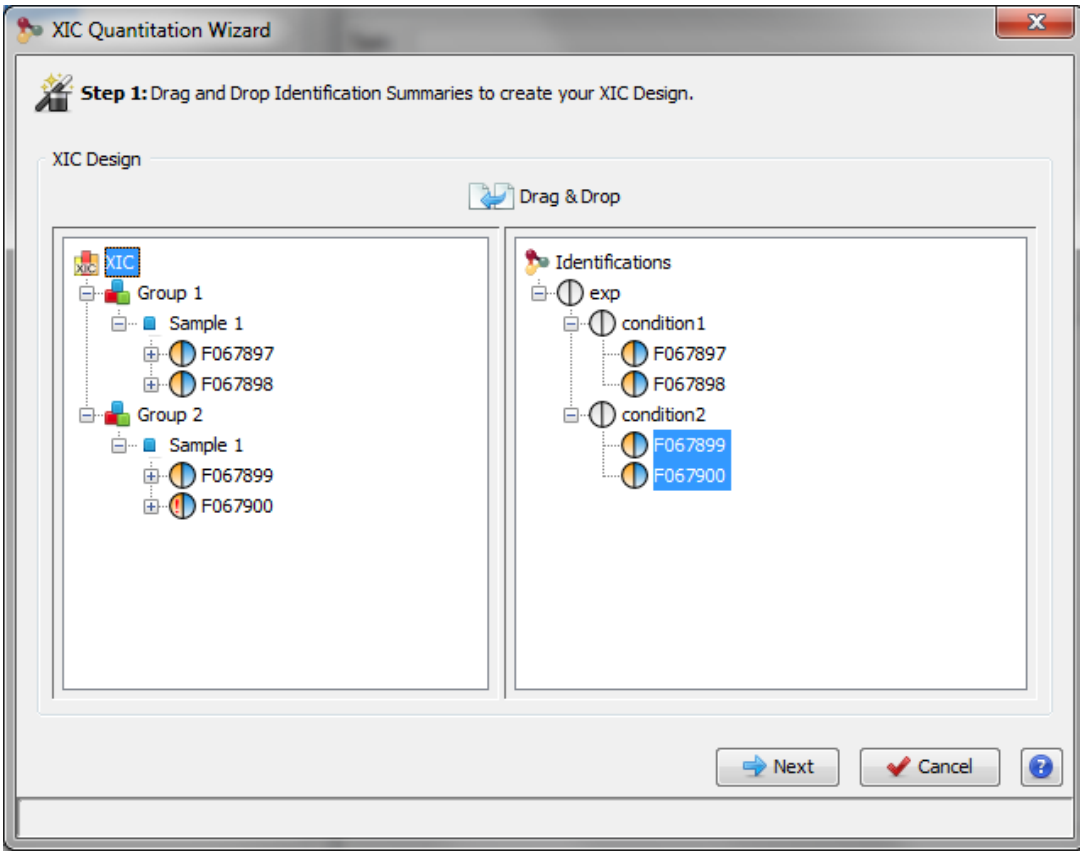


Create Design

To create the Design of your XIC, drag and drop the identifications from the right panel to the left panel.

If you drop an identification on the XIC Node, a Group and Sample parents nodes will be automatically created.

You can also directly drop on a Group or Sample Node.

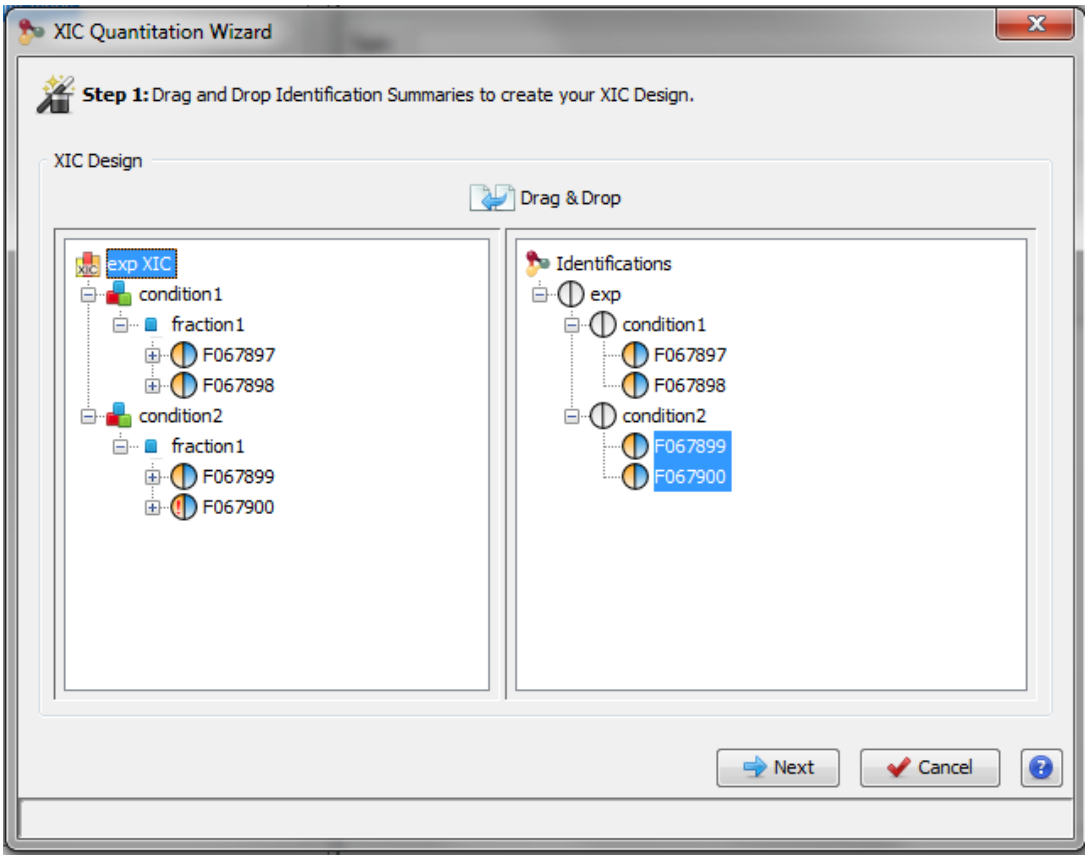


Rename Design

You can rename all the design nodes by different ways:

- by typing F2 when a node is selected
- by a long click on a node
- by the right button popup and the menu Rename

We recommend to rename at least the XIC node.

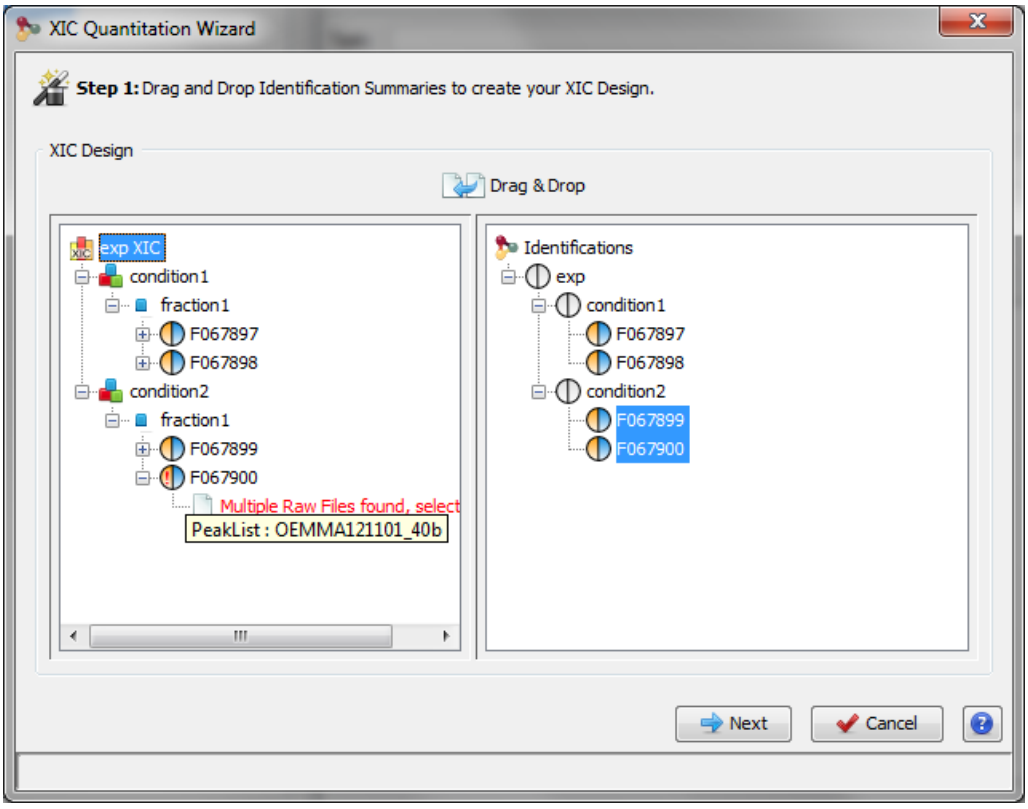


Link to Raw Files

To be able to perform a XIC design, we need to know the source raw files.

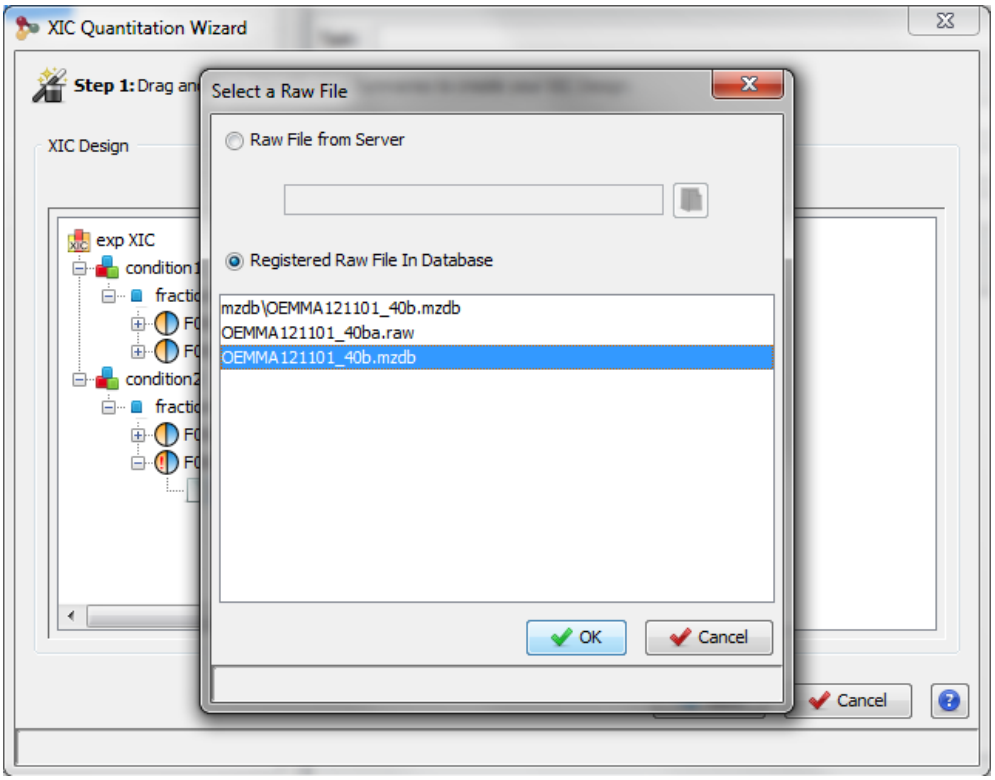
Proline try to find in the database the corresponding Raw Files already registered. If a Raw file is not found, the icon shows a '!' and you can display the error by expanding the corresponding node. In this case, you will have to select the Raw File by yourself.

Note: To help you, you can display the peaklist tooltip by overriding the Identifcation Node in the Design Tree.



To select a Raw File, click on the error, and use the following dialog.

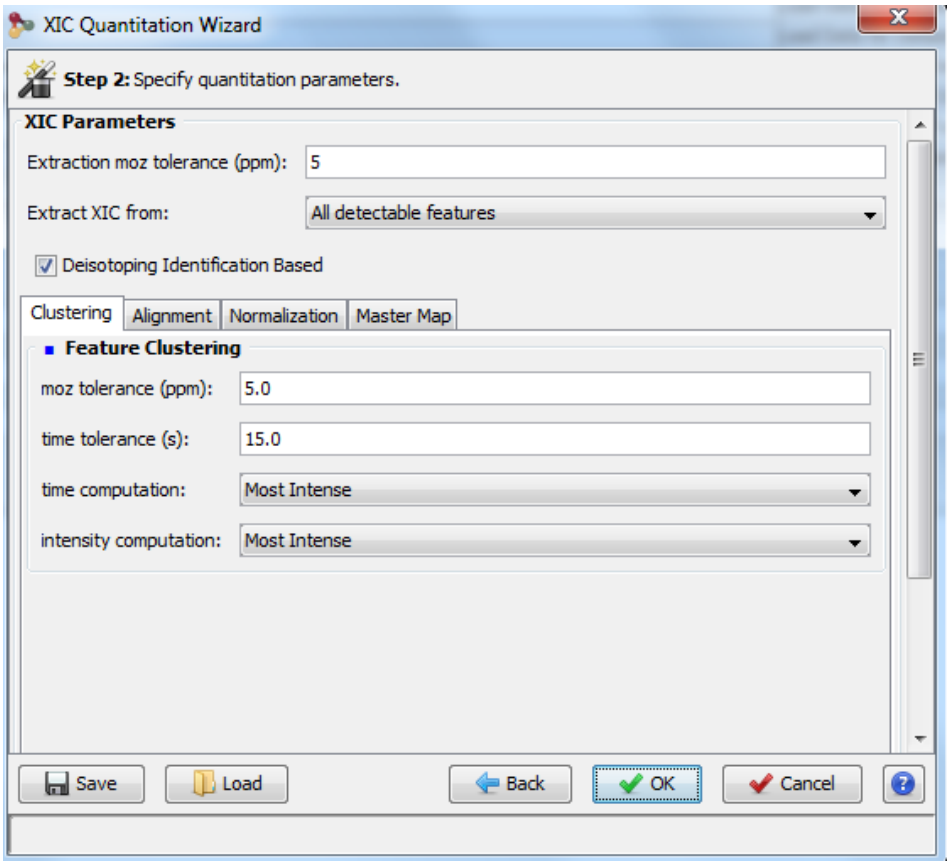
You can select directly a file on the disk or a potential corresponding Raw File registered in the database.



XIC Parameters

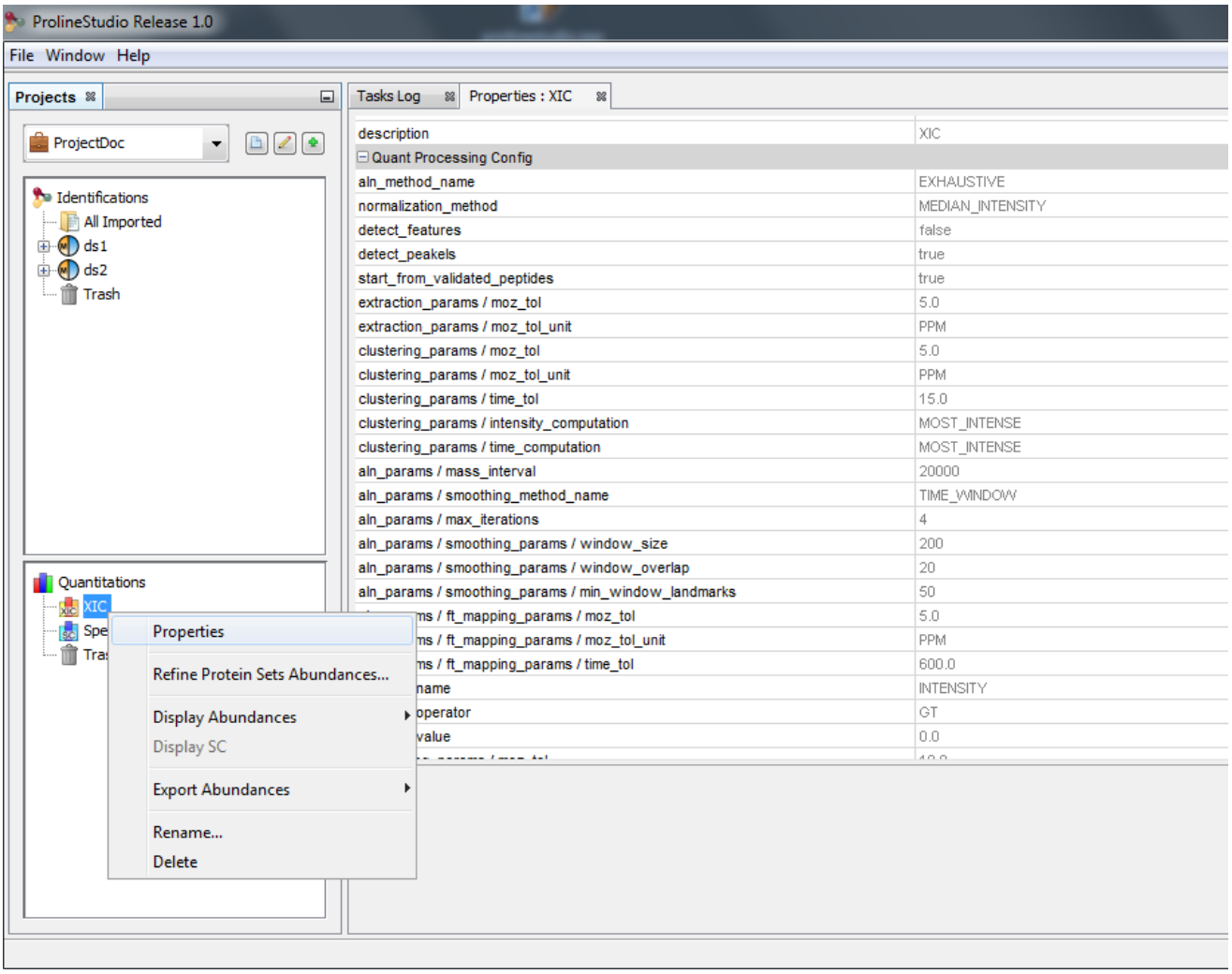
When the XIC Design is finished, click on the next button and select the parameters. See Label-free LC-MS quantitation configuration to have more details about the different parameters.

Note: all the parameters are already set with default values.



XIC Results

When the XIC Design has been generated, it is added in the Quantitation Tree. You can display its properties, especially the configuration used, by the right mouse button popup:



You can delete a XIC Design, see how to Delete Data

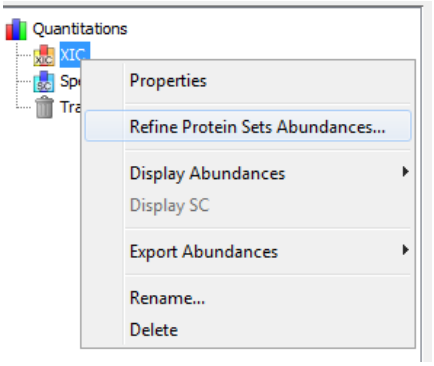
You can rename a XIC Design, by clicking on “Rename…” in the popup menu.

You can export the XIC results, see how to Export a XIC

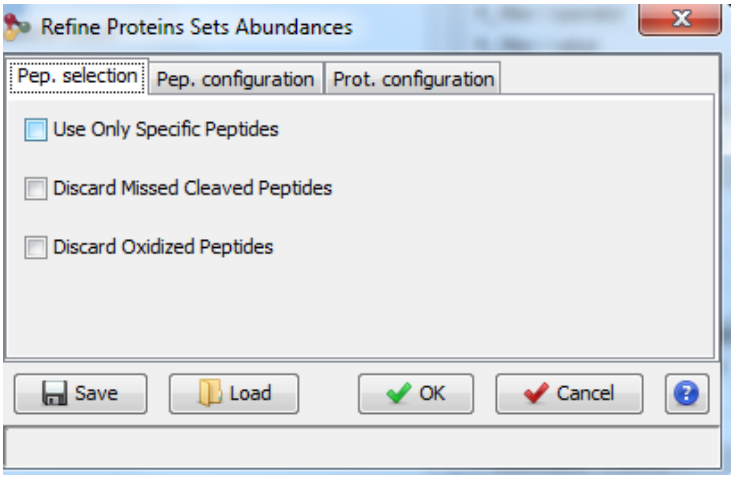
Refine Protein Sets Abundances

Advanced Protein Sets abundances

Right click on the selected XIC node in the Quantitation tree, and select “Refine Proteins Sets Abundances…”



Configuration



In the dialog, you can:

- specify peptides to consider for quantitation

- configure parameters used for peptides quantitation

- configure parameters used for proteins quantitation

For more details, see Post-processing of LC-MS quantitative results

Advanced XIC results

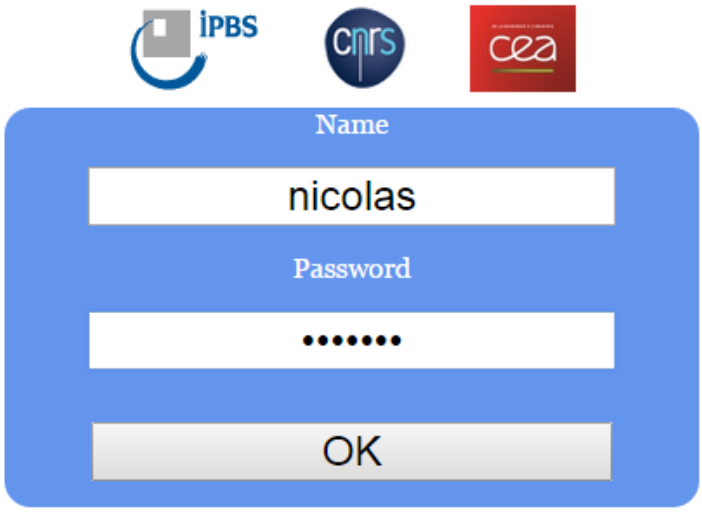
You can see the results by displaying the XIC (Display a XIC) or export them (Export a XIC)

Proline WEB

Server Connection

Prerequisite: You must have an account to login to the server. Ask your administrator to create one if you don't have any. **After the installation, the default account is "admin" with password "admin"**

- Open your Google Chrome web browser and connect to the address of the server (ask your administrator)

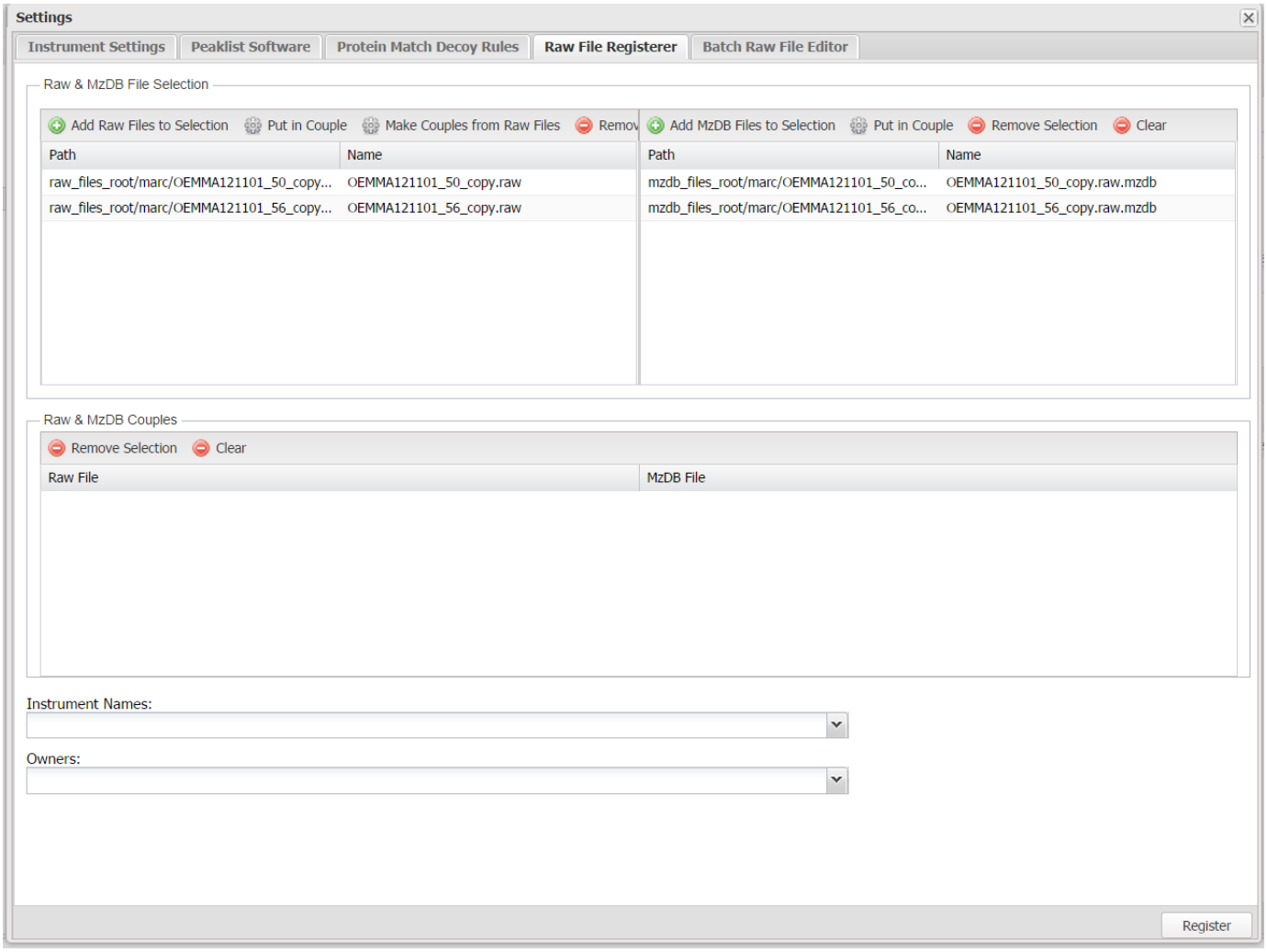


- Enter your username and password and click "OK".

- To create a project, please follow the instructions detailed on this page.

Register Raw & MzDB Files

In order to create and run Quantitation analyses, you must register your RAW files and corresponding MzDB files into Proline databses to do so, click on the "Settings" button in the top bar of the Dataset Explorer application, and go to the "Raw File Registerer" tab.



- In the left grid, use the “Add Raw Files to Selection” button in order to select Raw Files
- In the right grid, use the “Add MzDB Files to Selection” button in order to select MzDB Files
- You can then make automatic pairs, based on file names, by clicking on the “Make Couples from RAW Files” button. This will automatically add matching files into the “Raw & MzDB Couples” grid.

If you want to pair files whose names differ, you can proceed as follows:

- Select a file, either Raw or MzDB, in its grid,
- Click on the “Put in Couple” button,
- Select it in the “Raw & MzDB Couples” grid,
- Select the corresponding file of the other format (RAW or MzDB) in its grid,
- Click on its “Put in Couple” button.

After this, you must choose an Instrument Name and select the owner of the file in the users list.

Finally, click on “Register”. Note that this operation will fail if one of your raw file has already been registered.

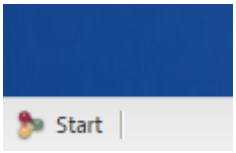
Close the Settings Window.

Installation Of the "Dataset Explorer" Application

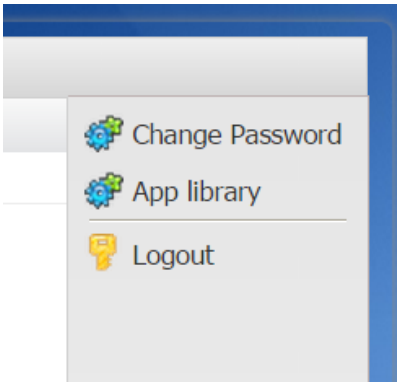
The Proline Web Desktop works like your Operating System : you need to install applications before you can use them.

In order to install the **Dataset Explorer** application, please proceed as follows :

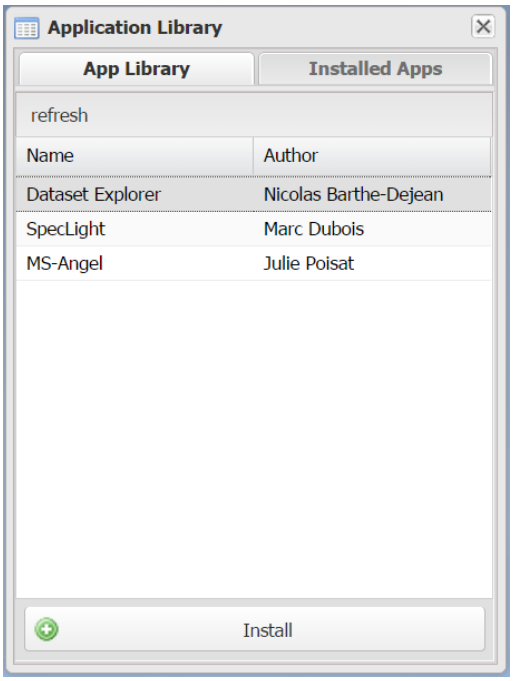
- click on the “Start Button” in the bottom-left corner of the page,



- click on “App Library” to open the Application Installation Menu

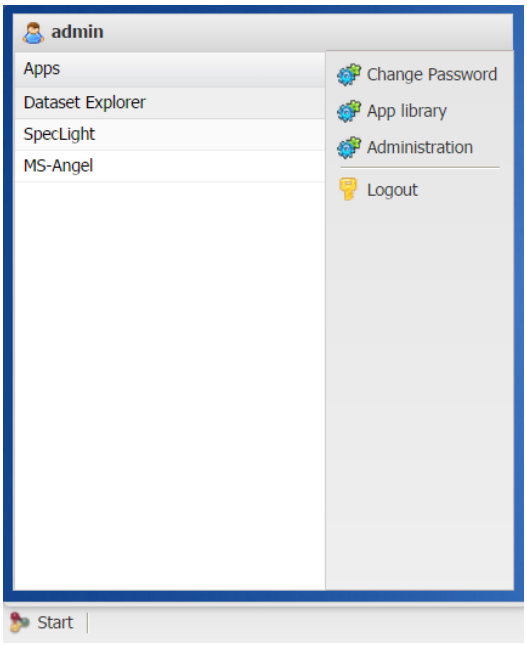


- select the “Dataset Explorer” Line and click on “Install”

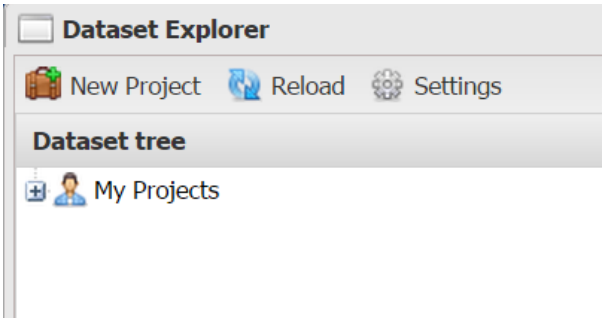


- A message should inform you that the Application has been well installed, and it should now appear in your start menu

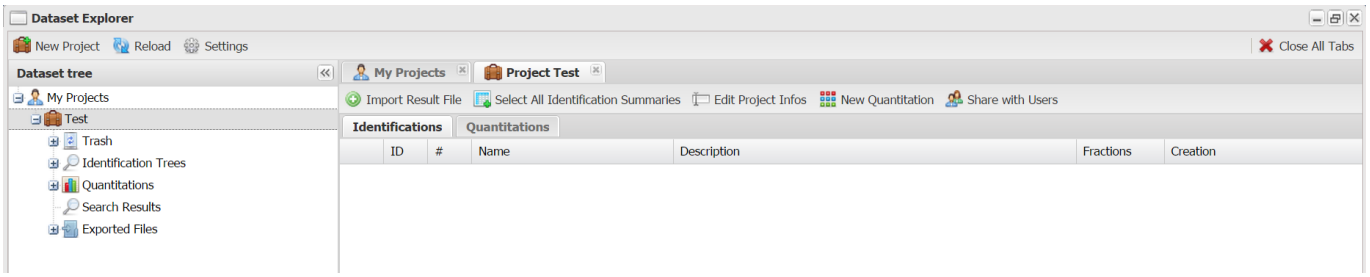
- Open it from the start menu by clicking on it



- Click on “New Project” and set a name and a description (optional) for it

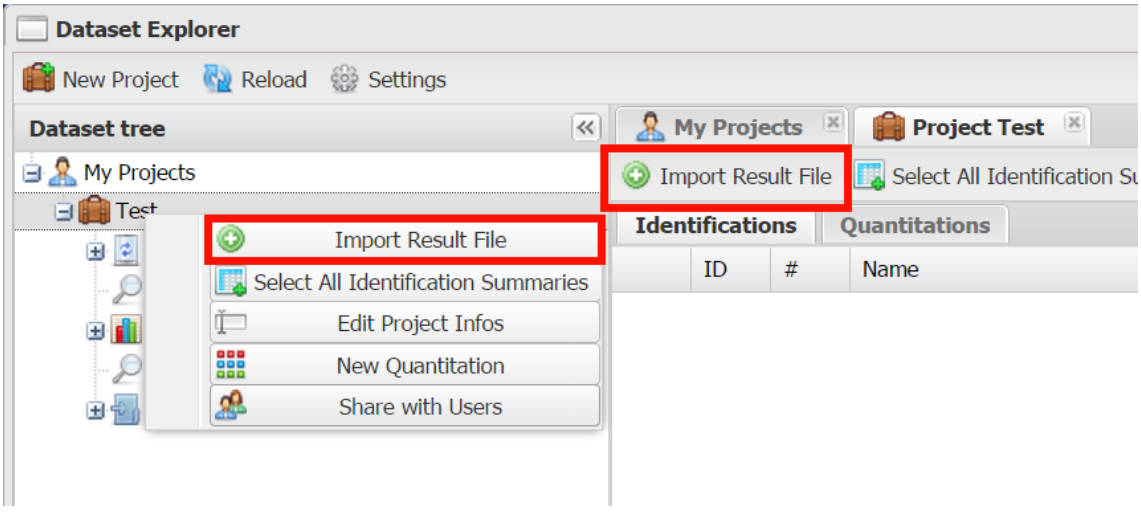


- Once your project is created, you can see it in the tree on the left of the screen. Click on it to make its options panel appear.

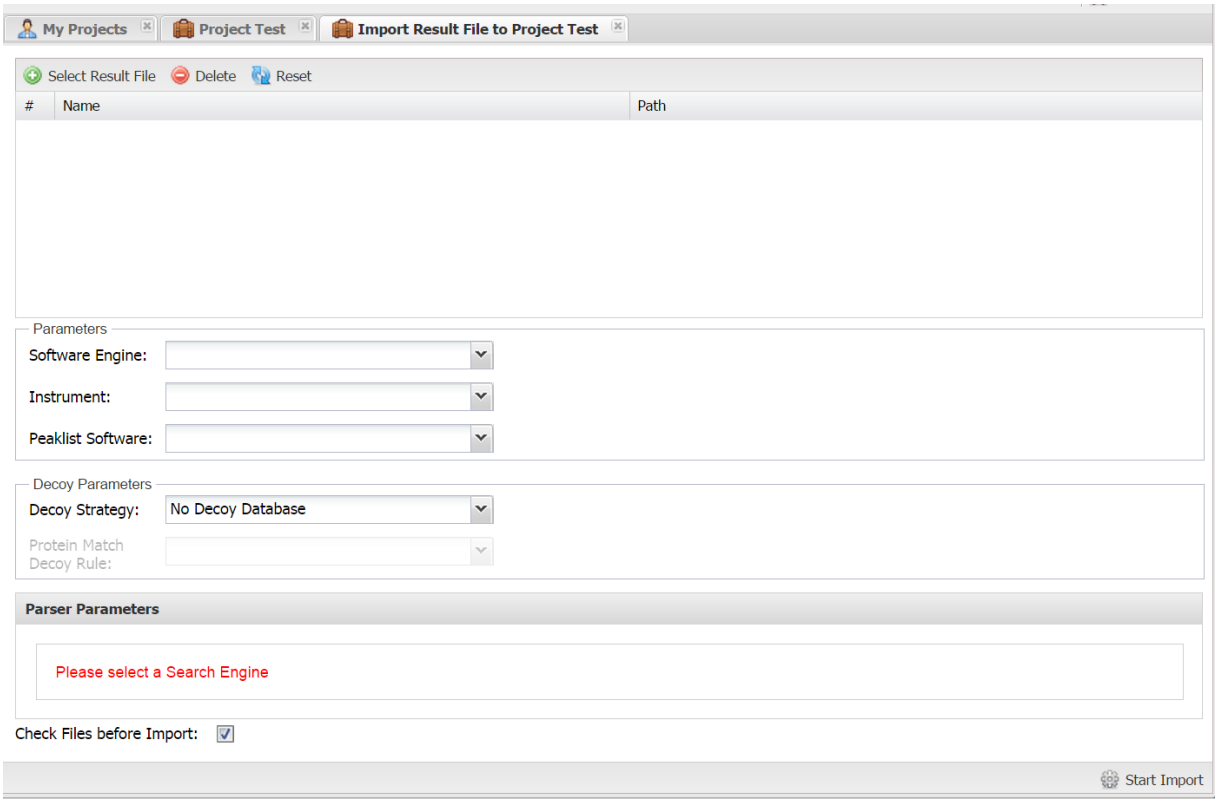


Import Result Files

The first thing to do in your brand new project is to import Result Files. To do so, Click on the “Import Result File” Button, in the toolbar of the project overview window or via a “right-click” on the Project node of the tree



You should then see this panel appear:



It allows you to select Result Files and set up following parameters for the import process:

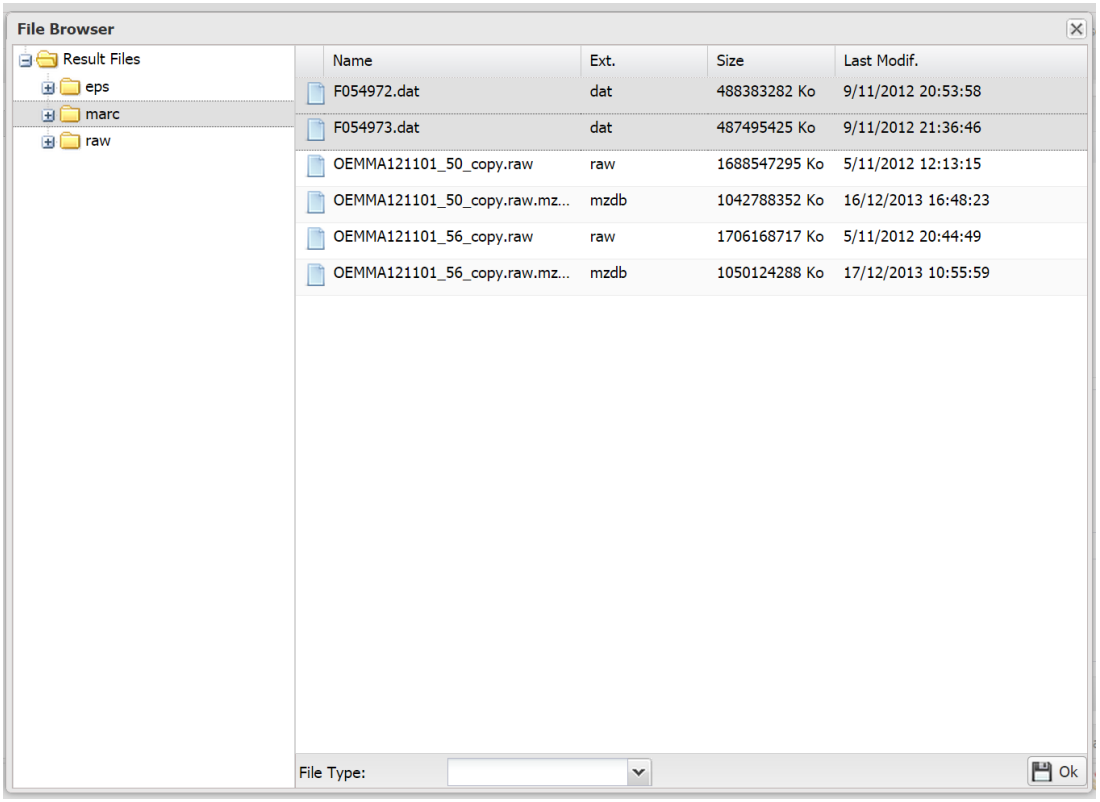
- Parameters
  - Software Engine : the software which generated your interrogation file
  - Instrument : mass-spectrometer used for sample analysis
  - Peaklist Software : the software used for the peaklist creation

- Decoy Parameters
  - Decoy Strategy : TODO
  - Protein Match Decoy Rule : TODO

- Parser Parameters: according to your Software Engine, this will display some extra-parameters.
  - Mascot :
    - Ion Score Cutoff : TODO
    - Subset Threshold : TODO
    - Mascot Server URL : TODO
  - Omssa :
    - User mod file : TODO
    - PTM Composition File : TODO

- Check Files before Import: let this checked to ensure that your files contain no errors. The server will perform a check operation before launching the import.

In order to add files to your import selection, click on “Select Result File” to open the File Browser that will let you choose one or many result files to import:

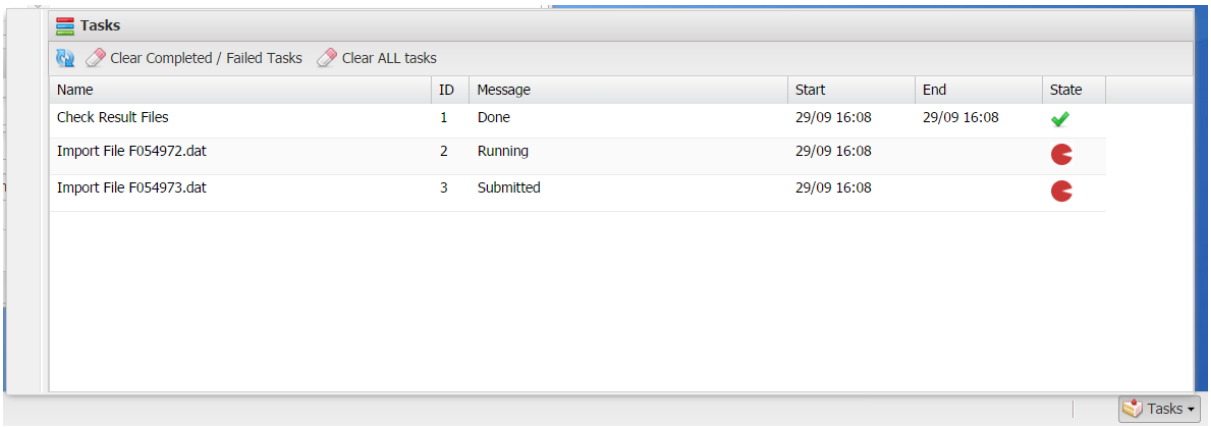


The left side let you browse the directories, and when you click on one of them, its content is shown on the main panel. Choose one or multiple files then click “Ok”.

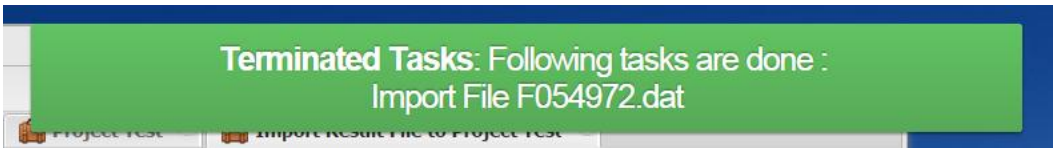
Back to the “Import Result File” window, you should now see your selection appear in the grid. Choose the instrument and the peaklist software corresponding to your files, and then select a “Decoy Strategy”. You can now click on “Start Import” to launch the check and the import tasks.

The server will check your files first, then the import itself will be launched automatically.

You can follow the current state of your tasks by clicking on the small cake in the bottom right corner of the desktop screen. It opens a small grid where you can see all your tasks.



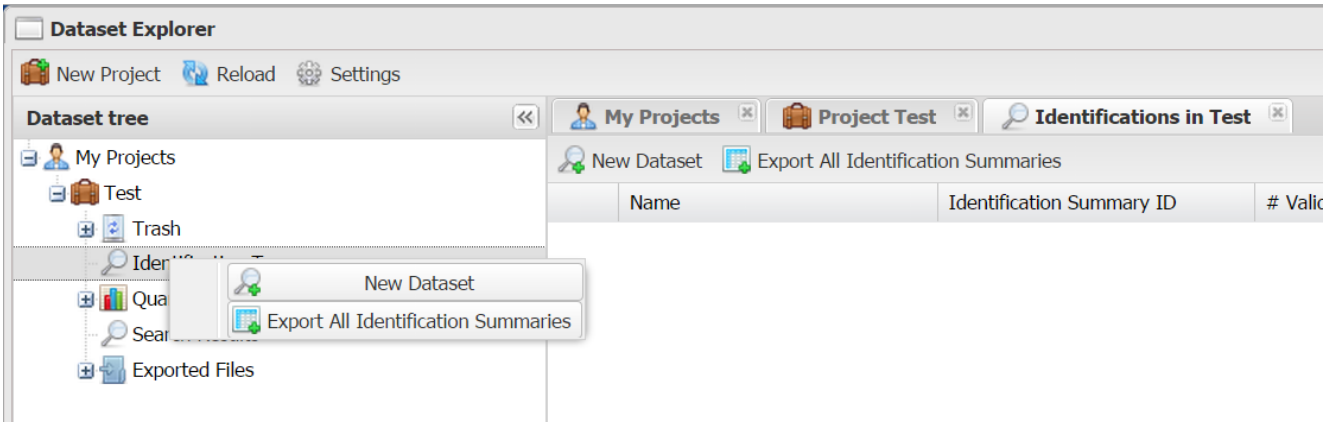
When a task is done, you are notified by a small message in the top of the screen, and you can see its status in the tasks window:



All the Result Files you have imported are listed in the “Search Results” panel you can access by double-clicking the “Search Results” node in your project's tree.

Create a new Dataset

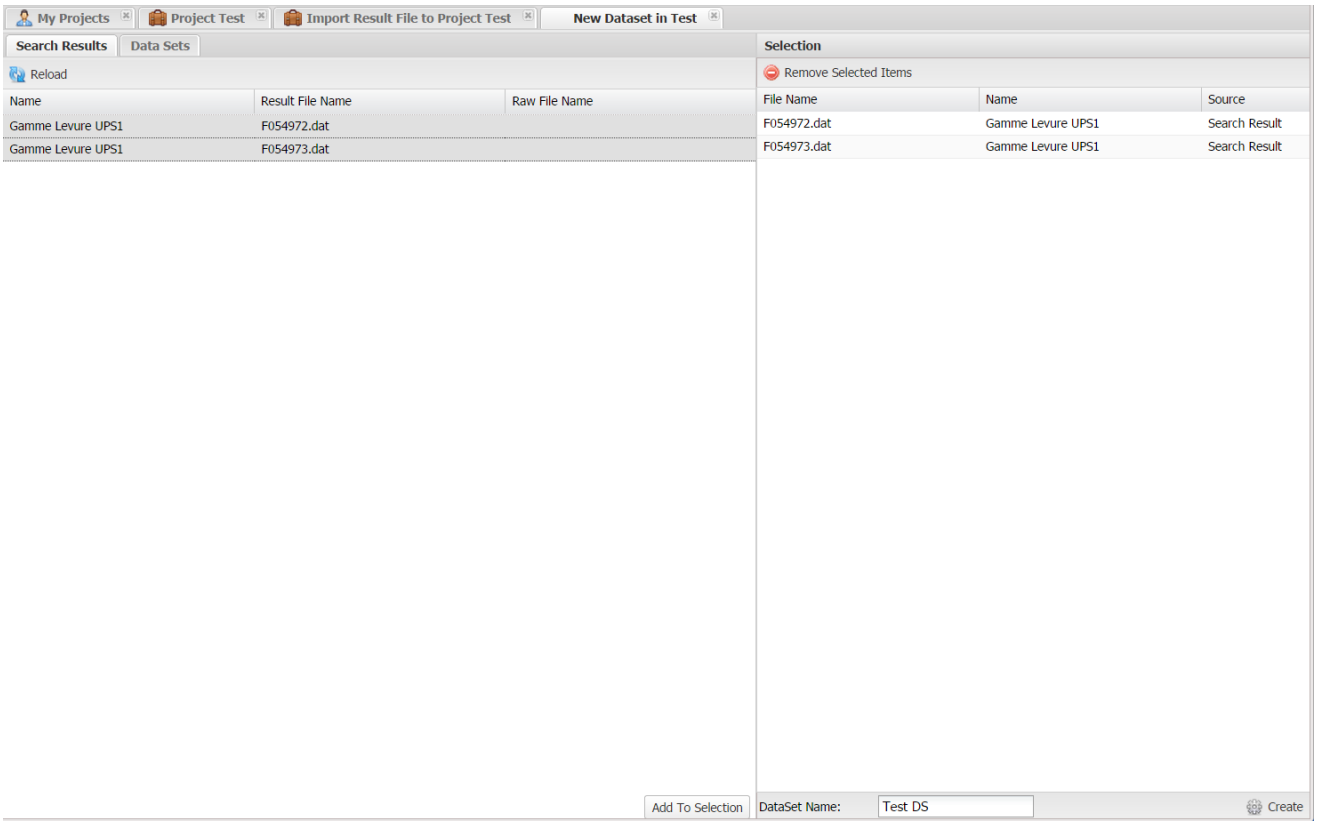
Once your Result Files have been imported, you can use them to create a new Identification Dataset. Double-Click on the “Identification Trees” element in the tree, on the left side of the window. The grid which just appeared is meant to list all your datasets. For now, click on the “New Dataset” button from the bar, or by right-clicking on the “Identification Trees” node.



You should now see a window asking to choose a source of data for your new Dataset.

- Choosing “Result Set” allows you to build a new dataset from both Result Files you have imported and existing identification datasets, whether they have been validated or not.
- Choosing “Result Summary” will let you build a new dataset from one or more existing validated identification dataset. Il will duplicate them into a new Dataset without their validation data.

For now, assuming you are creating your first identification dataset, you should choose the “Result Set” option. On the two-tabbed panel, go to the “Result Sets” tabs in order to see the list of the files you have imported.

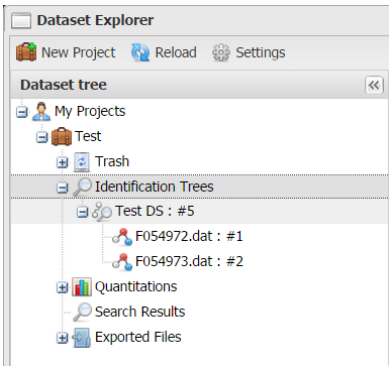


To add one or many files to your selection, select them in the grid (you can use the Ctrl and the Shift keys to make a multiple selection), then click on “Add to Selection” on the bottom of the window. You can also double click on one file to quickly add it to the selection.

To remove any file from the selection, just select them and click on “Remove selected Items”. Type a name for your dataset then click on “Create”. The creation of your identification dataset happens as follows:

- An “Aggreagation” Dataset Node is created. It takes the name that you provided during the creation.
- One “Identification” Dataset Node is created for each one of the Result Files you have selected. They take the name of the Result File.

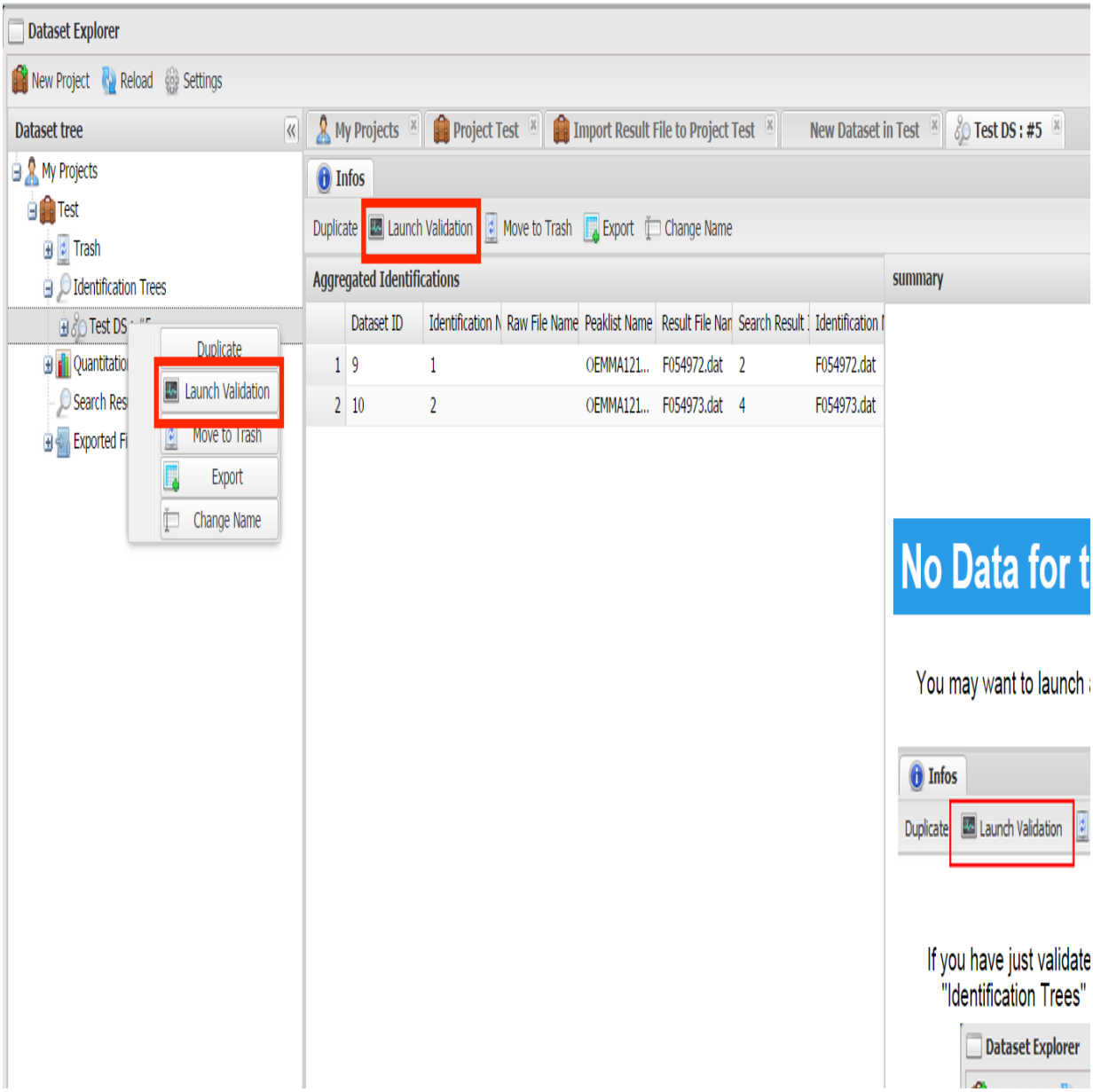
Once your Identification Dataset has been created, you can see it on the tree, in the left side of the window.



The panel of the Aggregation Node shows a list of your Identification fractions (corresponding to each imported file) and, after the validation process, it will display the Merged Result Summary infos.

Validate Search Result

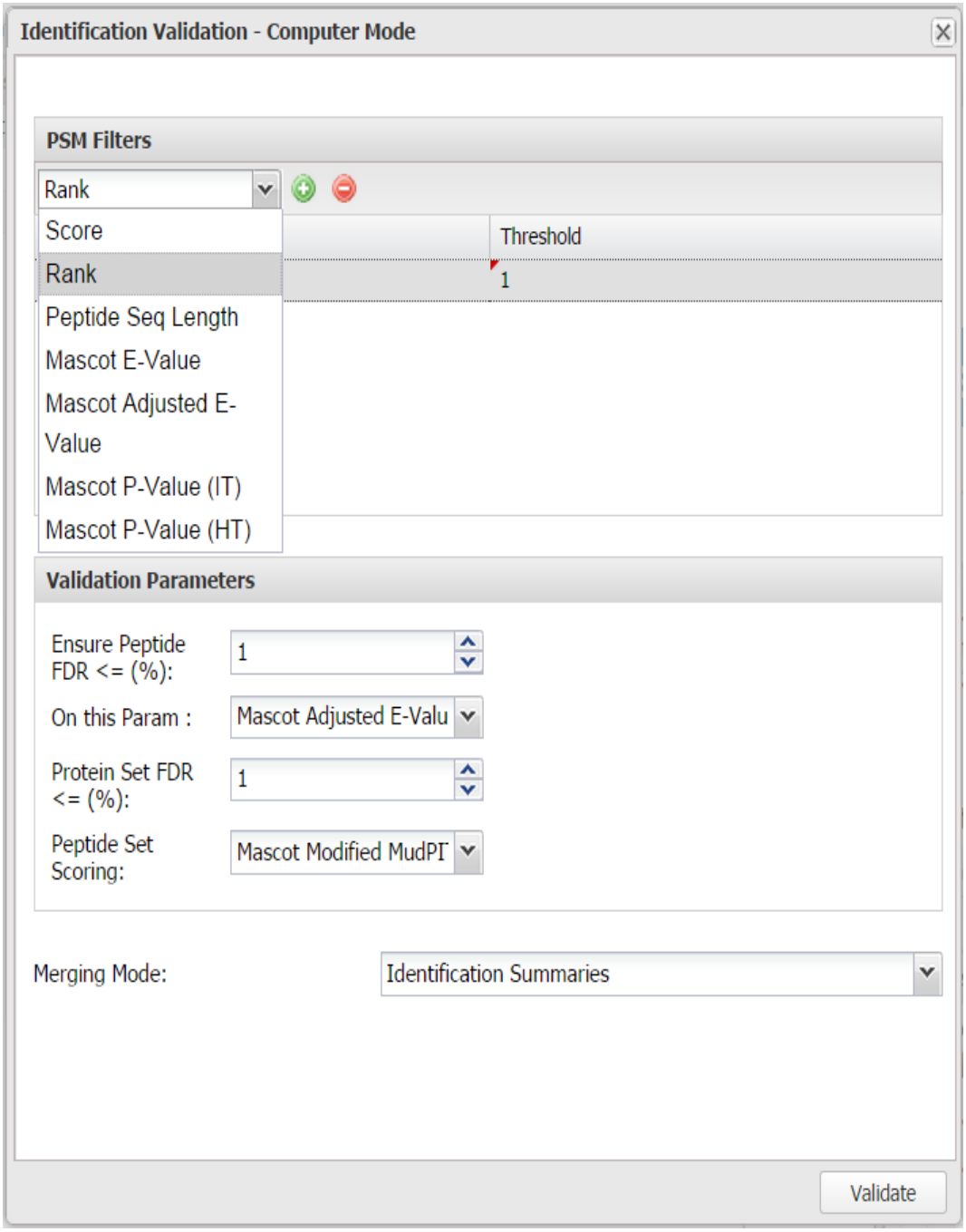
To launch a validation on a dataset, click on its node in the Project Tree on the left side of the Dataset Explorer.



Click on “Launch Validation” in the toolbar of the Infos tab.

You can also right click on the dataset node and click on use the “Launch Validation” button.

The following form appears:



The Validation form handles several settings:

- Merge Search Results: choosing “YES” will merge the Result Sets (corresponding to your result files) before launching the validation of the merged dataset. “NO” will validate the result sets separately and merge their result after the validation process.

- Filters: let you filter the data that will be included in the validation results. For example, you might want to keep only the peptide of rank 1. To add a filter parameter, choose a setting in the selection box, click on the “+” button, then edit the threshold value of the parameter on its line.

- Validation Thresholds let you define the False Discovery Rate of your peptides and proteins. You must define from which parameter the Peptide FDR should be estimated:

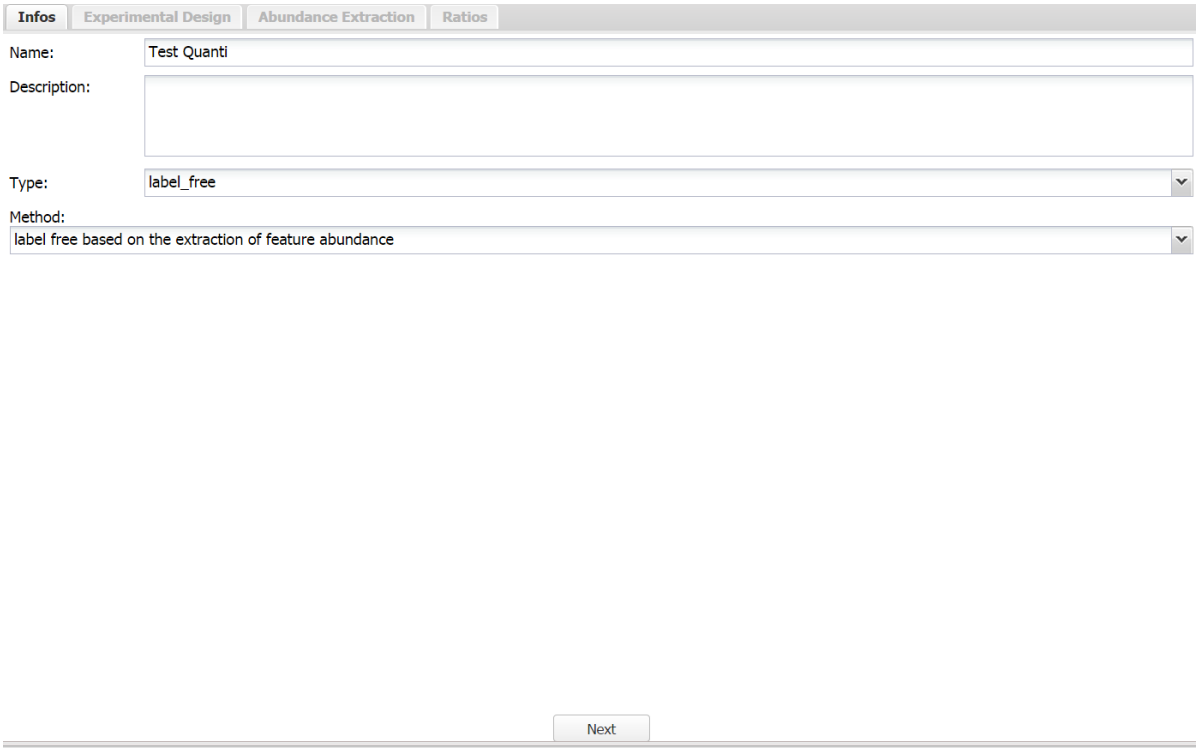
When ready, click on “Validate” to launch the validation task. You can see it in the tasks panel.

Create a Quantitation

Double-click on the “Quantitations” node of your project tree to show you Quantitations table panel. It's empty if you haven't created any quantitation in this project yet. Click on the “New Quantitation” button to open the Quantitation Creation Panel.

Note that you can also open it by right-clicking on the Quantitation node or on the Project node itself.

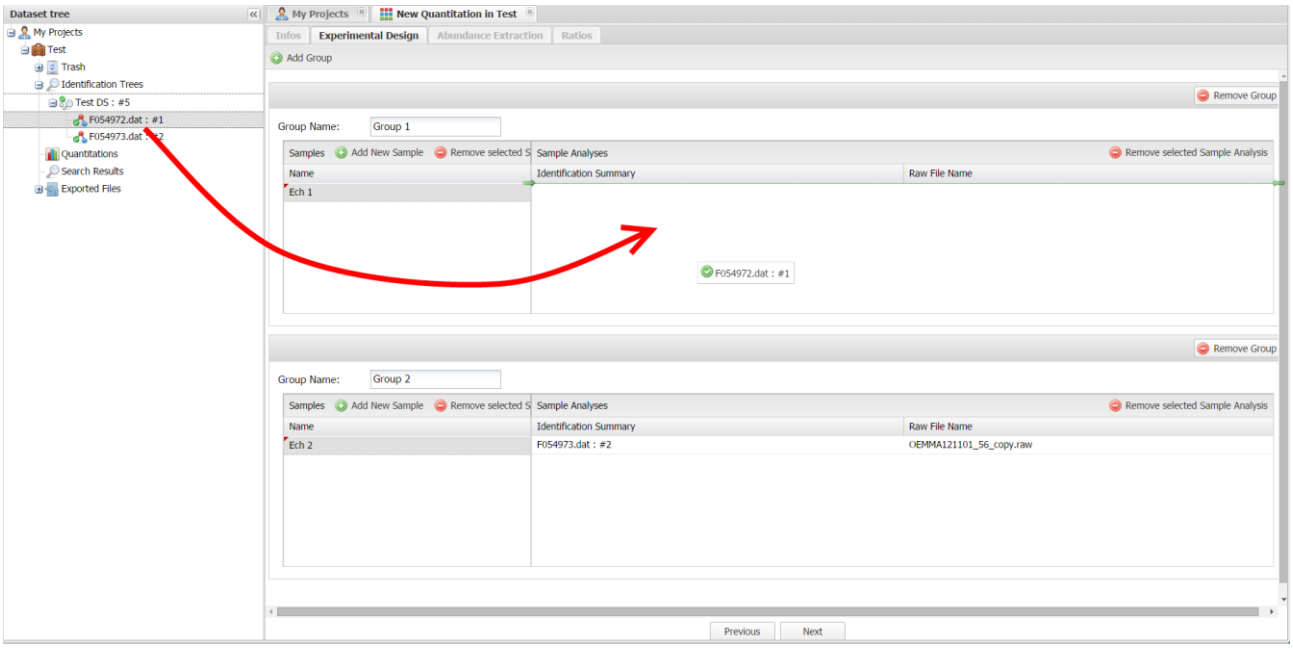
Title, Type and Method



The first tab of the Quantitation creation panel let you define a name, a description (optional), choose a type and a method.

Once you've made your choices, click on “Next”.

Experimental Design



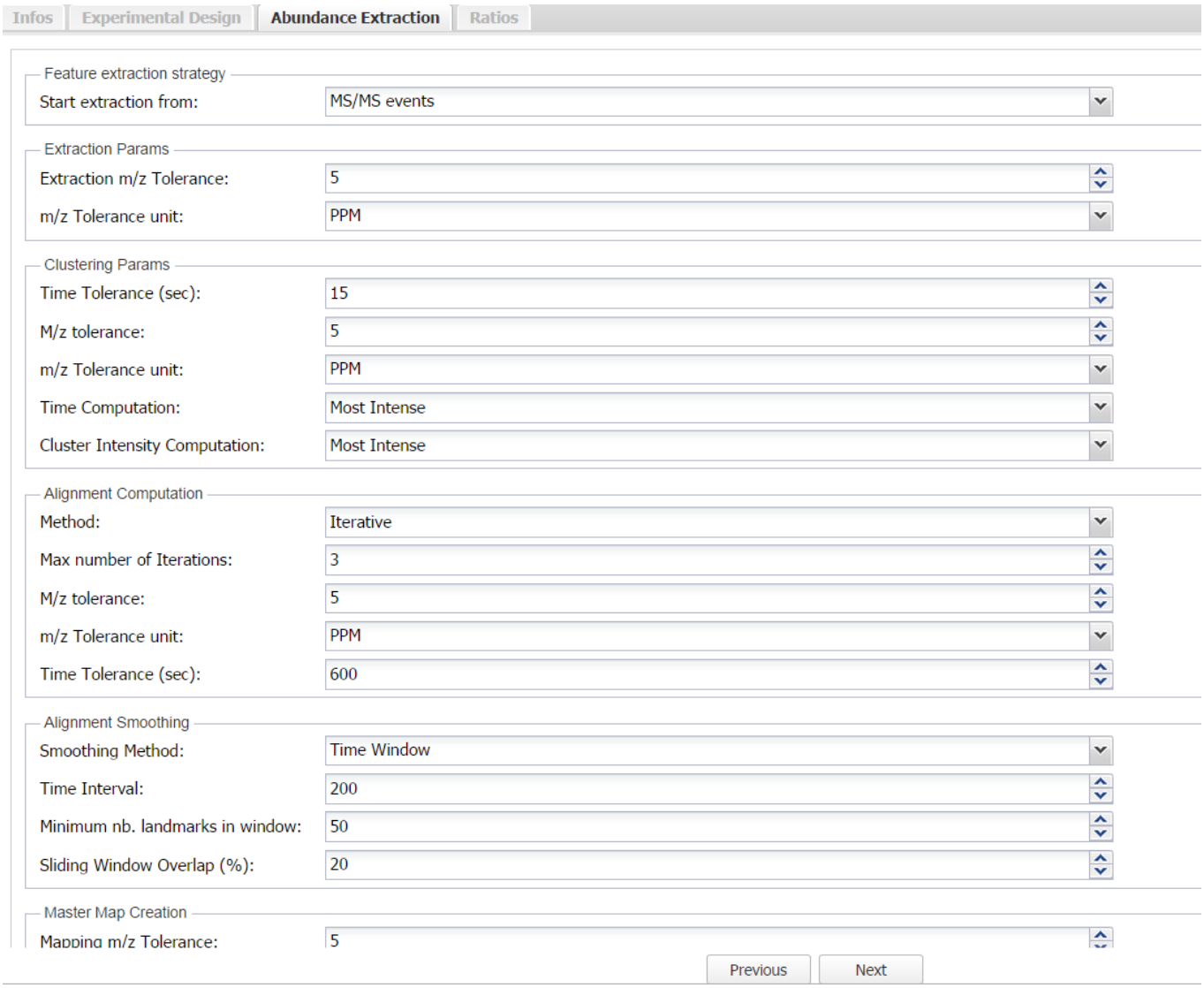
The Experimental design tab is where you define your Groups and Samples. By default, two groups are created and each one contains a sample.

You can create new groups by clicking on the “Add Group” button in the top bar of the tab. In each group, you can manage your sample by using the buttons in the left grid.

To add a Search Result to one of the samples of the group, select it in the left grid, and **drag and drop a validated result set from your project's tree to the “Sample Analyses” grid**

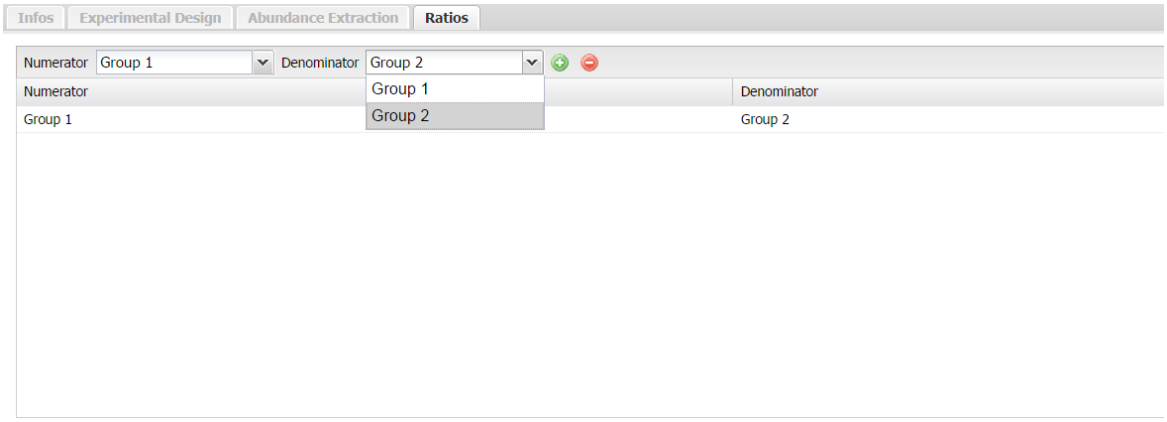
Once you have prepared all your group and samples, click on the “Next” button.

Abundance Extraction parameters



This tab let you set up you abundance extraction parameters.

Ratios



The purpose of this tab is to define the ratios between the groups your quantitation will rely on

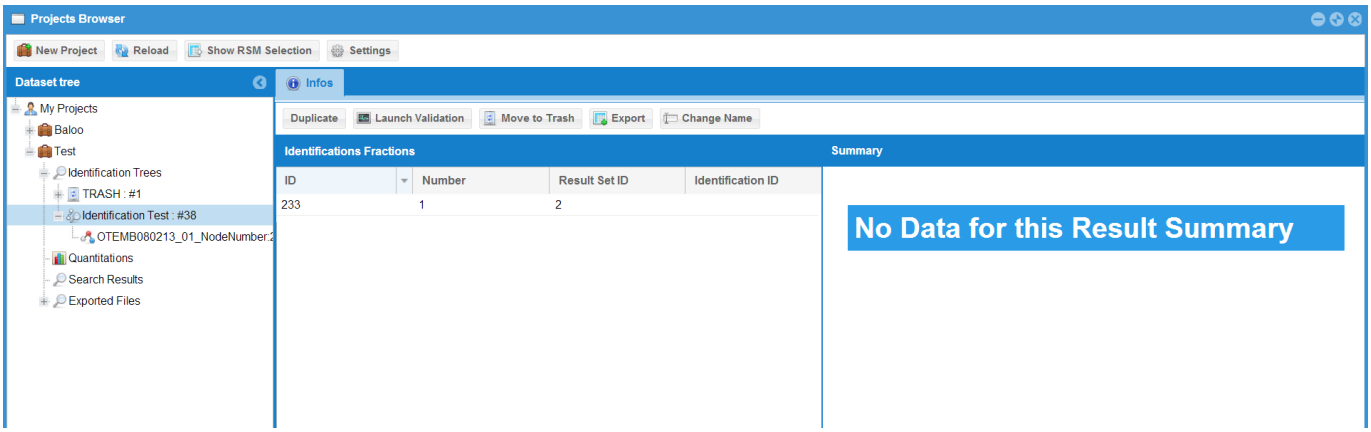
Launch the Analysis

When you're done, just press the “Launch Quantitation” button. You will be noticed when the task is finished

---

Delete Datasets

You can delete an Identification dataset by clicking on it on the Dataset Explorer left side panel, and then click on the “Move to Trash” button in the toolbar of the “Infos” tab.



You can also right click on the dataset you want to delete, and click on the “Move to Trash” button.

All deleted datasets are visible in the “Trash” node in the project tree.

Create a User

You must be logged in as an administrator.

Click the “Start” button, go to “Administration”. On the first tab, “User Administration”, you can create a new user by setting up its name, its password, and define whether or not the user will have the “administration” permissions (including applications, users and service management).

Submit the form to create the user. You can manage existing users from the “users” tab.

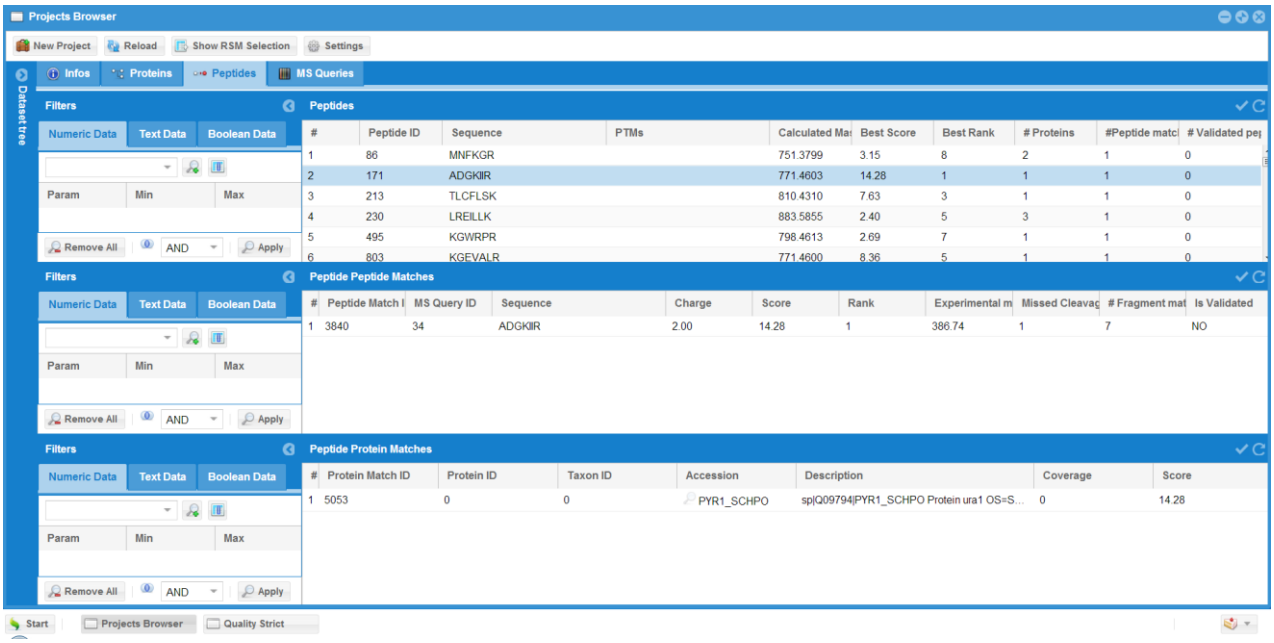
Please note that the Proline Web Desktop has its own database and its own users collection. However, if you configured the Proline Service running inside the Proline Web Desktop, it will synchronise the Proline Web Desktop and the Proline Core users each time a user logs in the Proline Web Desktop:

- the users you create in the Proline Web Desktop Administration panel will be automatically added to the Proline Core database (User Data Set database) when they sign in on the Proline Web Desktop.
- the Users registered in the Proline Core Database (UDS) will be automatically registered in the Proline Web Desktop database when you log in as administrator in the Proline Web Desktop, if they were missing.

Peptides Table

The “Peptides” tab of a validated Identification Dataset (or Merged Dataset) allows you to browse the peptides of the related Result Summary.

Each table of the Result Summary data viewer provides a set of Filters for Numerical, Text and Boolean data, placed on the left of the grid.



Peptide Click Actions:

- Clicking on a Peptide will automatically display the peptides matches related to the selected peptide on the Peptide Matches table, and the Protein Matches table will display the protein matches related to the selected peptide.

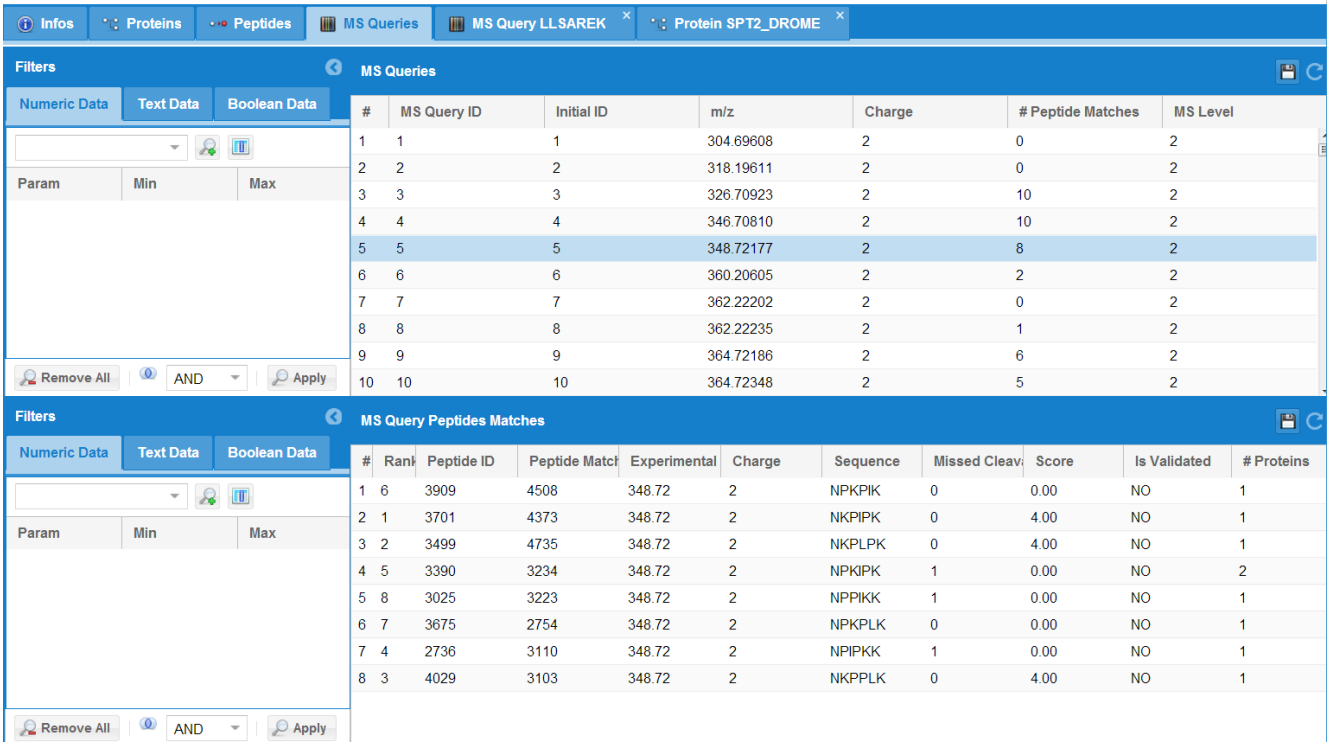
Double Click actions:

- If you double click on a peptide match, an MS Queries viewer tab focused on the corresponding ms query (a filter will automatically applied) will be opened.

- If you double click on a protein match, a Proteins viewer tab focused on the corresponding protein will be opened.

MS Queries Table

The MS Queries table displays the MS Queries of the Result Summary and offers the same filters options as the others tables.


Infos
Proteins
Peptides
MS Queries
MS Query LLSAREK
Protein SPT2_DROME
Filters
Numeric Data
Text Data
Boolean Data
Param
Min
Max
Remove All
AND
Apply
MS Queries
| MS Query ID | Initial ID | m/z | Charge | # Peptide Matches | MS Level
1 | 1 | 1 | 304.69608 | 2 | 0 | 2
2 | 2 | 2 | 318.19611 | 2 | 0 | 2
3 | 3 | 3 | 326.70923 | 2 | 10 | 2
4 | 4 | 4 | 346.70810 | 2 | 10 | 2
5 | 5 | 5 | 348.72177 | 2 | 8 | 2
6 | 6 | 6 | 360.20605 | 2 | 2 | 2
7 | 7 | 7 | 362.22202 | 2 | 0 | 2
8 | 8 | 8 | 362.22235 | 2 | 1 | 2
9 | 9 | 9 | 364.72186 | 2 | 6 | 2
10 | 10 | 10 | 364.72348 | 2 | 5 | 2
Filters
Numeric Data
Text Data
Boolean Data
Param
Min
Max
Remove All
AND
Apply
MS Query Peptides Matches
| Rank | Peptide ID | Peptide Match | Experimental | Charge | Sequence | Missed Cleav | Score | Is Validated | # Proteins
1 | 6 | 3909 | 4508 | 348.72 | 2 | NPKPIK | 0 | 0.00 | NO | 1
2 | 1 | 3701 | 4373 | 348.72 | 2 | NKPIPK | 0 | 4.00 | NO | 1
3 | 2 | 3499 | 4735 | 348.72 | 2 | NKPLPK | 0 | 4.00 | NO | 1
4 | 5 | 3390 | 3234 | 348.72 | 2 | NPKIPK | 1 | 0.00 | NO | 2
5 | 8 | 3025 | 3223 | 348.72 | 2 | NPPIKK | 1 | 0.00 | NO | 1
6 | 7 | 3675 | 2754 | 348.72 | 2 | NPKPLK | 0 | 0.00 | NO | 1
7 | 4 | 2736 | 3110 | 348.72 | 2 | NPIPKK | 1 | 0.00 | NO | 1
8 | 3 | 4029 | 3103 | 348.72 | 2 | NKPPLK | 0 | 4.00 | NO | 1

MS Query Click:

- Clicking on an MS Query will automatically load the corresponding Peptide Matches in the “MS Query Peptides Matches” table

Double Click Actions:

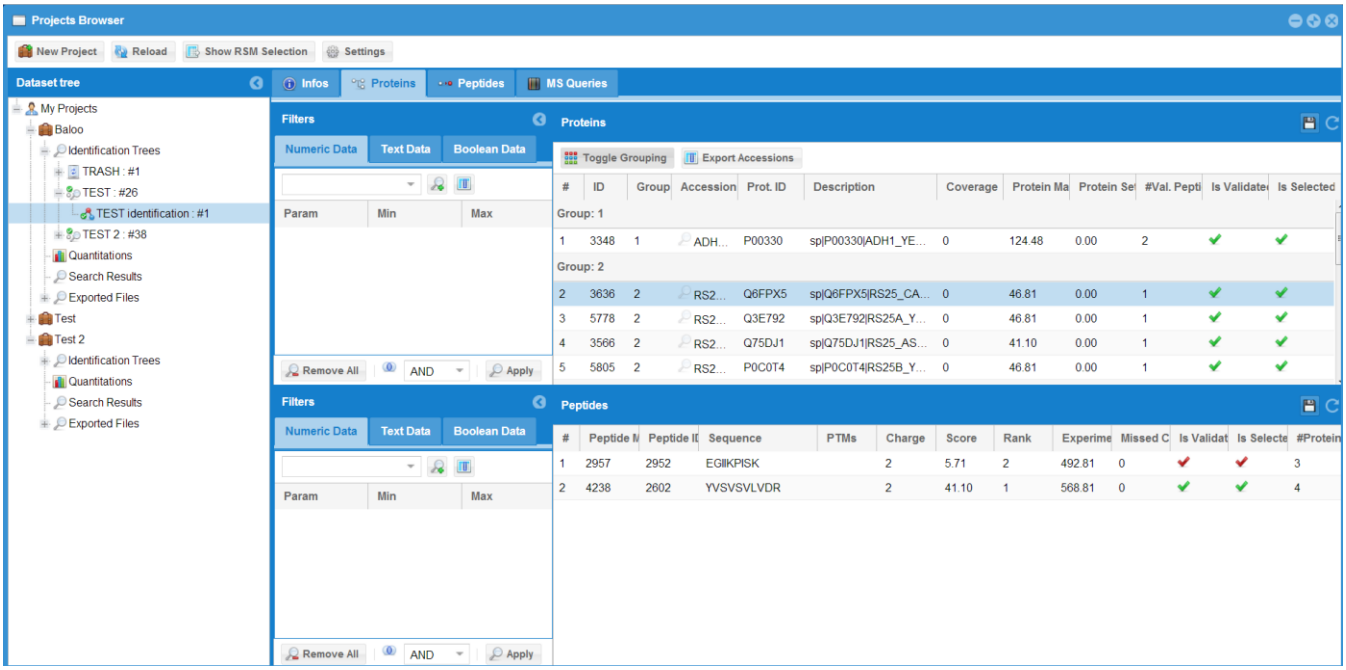
- Double clicking on a Peptide Match item will open a Peptides viewer tab focused on the corresponding peptide.

Display Proteins

In order to browse the protein set data of a validated identification, click on the validated identification node in the project tree, on the left side panel of the dataset explorer, and then open the “Proteins” tab.

Each table of the Result Summary data viewer provides a set of Filters for Numerical, Text and Boolean data, placed on the left of the grid.

IMPORTANT : Please note that the Protein Table only displays the validated proteins. You can reset this filter by clicking on the “Remove All” button of the proteins table filter panel, or by clicking on the circle arrow in the upper right corner of the table.



Protein click action:

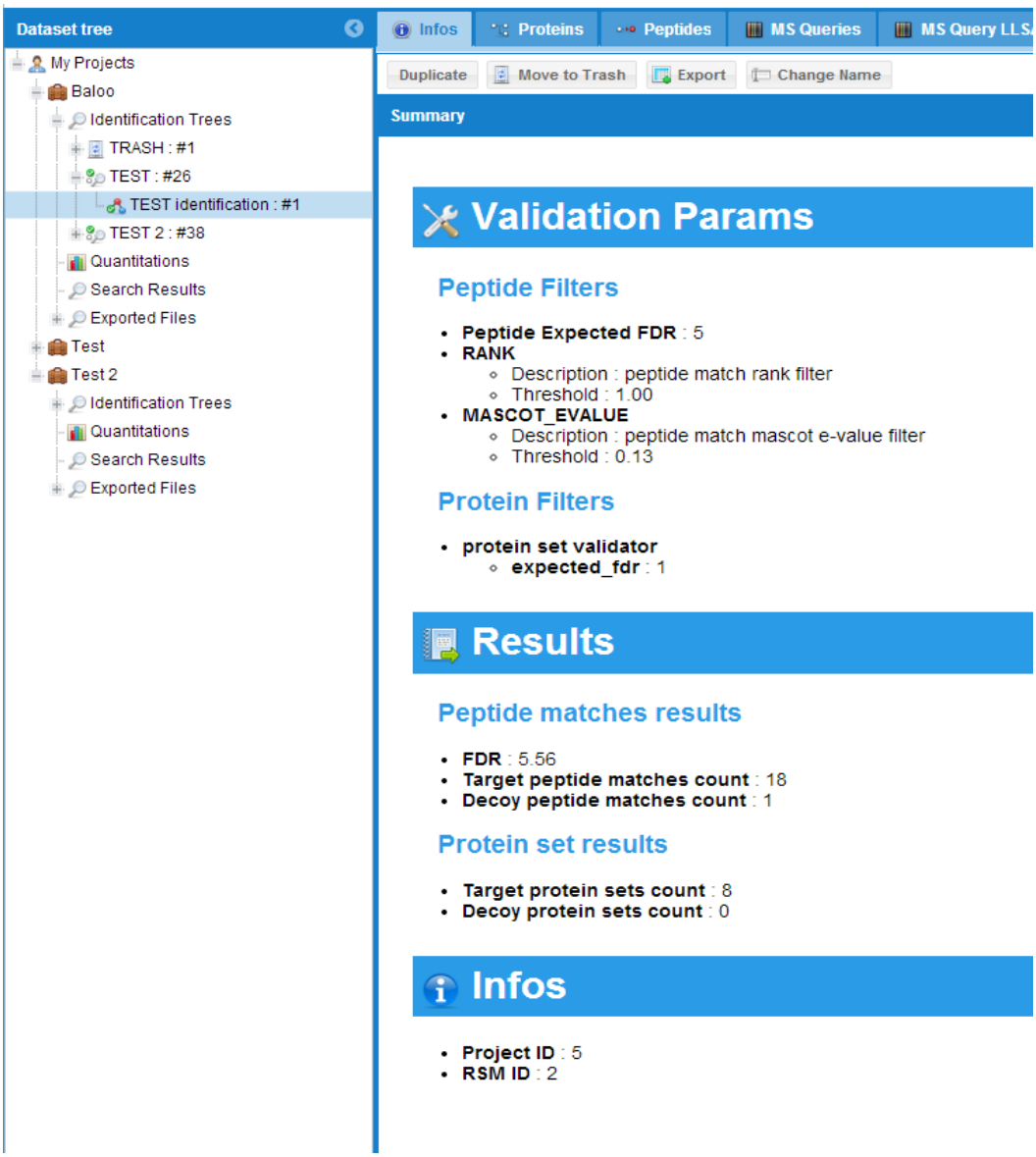
- Clicking on a protein will automatically load the related peptides in the bottom table. - Clicking on the small Magnifier near the AC Number of a protein will open the UniProt app (if you have installed id) focused on the corresponding protein.

Double click actions:

- Double Clicking on a peptide of the Peptide table will open a new Peptide viewer tab focused on this peptide.

Display Identification Summary additional information

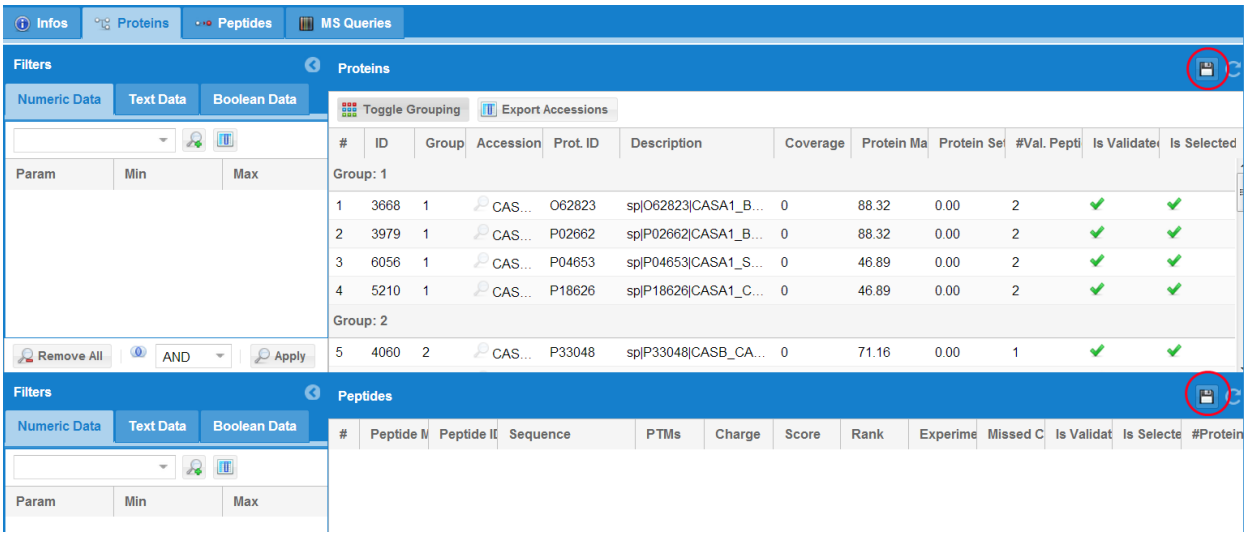
The “Infos” panel sums up the validation parameters and results.



If you clicked on an Aggregate node, this panel will show the infos of the Merged Result Summary and a grid listing all of the identifications of this aggregate.

Export Data

To export any of the Result Summary data table, just click on the save icon on the top of the table. Please note that any current filter applied to the table will be applied to the exported data.



Raw file conversion to mzDB

raw2mzDB installation

1. get the zip archive
2. installation of MSFileReader from Thermo (here, will install all necessary c++ redistribuables)
3. Ensure your regional settings parameters are '.' for the decimal symbol and ',' for the list separator

Use case procedure

Open a command line window in the directory containing raw2mzdb.exe
Type:
raw2mzdb.exe -i <rawfilename> -o <outputfilename>
By defaut, the raw file will be converted in the "fitted" mode for the MS1 (MS2 is often in centroid mode and can not be converted in fitted mode). If the MS2 (or superior) are acquired in high resolution (i.e in profile mode), you could specify that you want to convert several MSs in the required mode: **raw2mzdb.exe -i <rawfilename> -o <outputfilename> -f 1-2** will try to convert MS1 to MS2 in fitted mode.

There are two other available conversion modes:

1. "profile", the command line is then: **raw2mzdb.exe -i <rawfilename> -o <outputfilename> -p 1** (means you want profile mode for MS1, others MS will be stored as they were stored in the raw file)

2. “centroid” : **raw2mzdb.exe -i <rawfilename> -o <outputfilename> -c 1** (means you want centroid mode for MS1, others MS will be stored as they were stored in the raw file)

CONCEPTS

Proline Concepts & Principles

- Dataset types:
  - Result File
  - Search Results
  - Decoy Searches
  - Identification Summary
- Data Processings:
  - Protein Inference
  - Protein and Proteins Sets scoring
  - FDR Estimation
  - Validation Algorithm
  - Merge multiple Search Results
  - Merge multiple Identification Summaries
  - Compare with Spectral Count
  - Quantitation (Principle)
    - LC-MS quantification
    - LC-MS quantification workflows
    - mzDB-processing
    - Label-free LC-MS quantitation workflow
  - Quantitation (Configuration)
    - Label-free LC-MS quantitation configuration
    - Post-processing of LC-MS quantitative results

- Data Import/Export:
  - Identification Summary Export

Result File

A **Result File** is the file created by a search engine when a new search is submitted. OMSSA (`.omx` files) and Mascot (`.dat` files) search engines are currently supported by Proline. A first step when using Proline is to import Result Files through Proline Studio or Proline Web.

Search engines provide different types of searches for MS and MS/MS data. It is important to highlight that the Result File content depends on the search type. Thus, Mascot searches must be currently performed using MS/MS ions search in order to be properly imported by Proline. Peptide Mass Fingerprint and MS/MS error tolerant searches will be supported in further versions of Proline.

Search Result

A **Search Result** (aka ResultSet in the database schema) is the raw interpretation of a given set of MS/MS spectra given by a search engine or a *de novo* interpretation process. It contains one or many peptides matching the submitted MS/MS spectra (**PSM**, i.e. Peptide Spectrum Match), and the protein sequences these peptides belong to. The Search Result also contains additional information such as search parameters, used protein sequence data-bank, etc.

A **Search Result** is created when a Result File (Mascot `.dat` file or an OMSSA `.omx`) file is imported in Proline. In the case of a target-decoy search, two **Search Results** are created: one for the target PSMs, one for decoy PSMs.

Content of a Search Result

Importing a Result File creates a new Search Result in the database which contains the following information:

- Search Settings: software name and version, parameters values
- Peak-List and Spectrum information: file name, MS level, precursor m/z, …
- Search result data:
  - Protein sequences
  - Peptide sequences
  - Spectra
  - 2 kinds of Matches
    - Peptide Spectrum Matches, i.e. the matching between a peptide and a spectrum, with some related data such as the score, fragment matches…
    - Protein Matches, i.e. the proteins in the databank corresponding to the PSMs identified by the search engine

Mascot result importation

The peptide matches score correspond to Mascot ion score

OMSSA result importation

The peptide matches score correspond to the negative common logarithm of the E-value:

- Score = -log10(E-value)

Decoy Searches

Proline handles decoy searches performed from two different strategies:

- Concatenated searches:
  - A protein databank is created by concatenating target protein sequence to decoy protein sequence. Deoy could be created using reverse or random strategie. From Mascot or OMSSA point of view, a unique search is done using that databank.
- Separated searches:
  - Two searches are done using the same peaklist, one on a target protein databank and one on a decoy protein databank. These searches are then combinated to retrieve usefull information such as FDR … Mascot allows user to check a decoy option and will automalically create a decoy databank.

Decoy and Target Search Result

- Concatenated searches:
  - When importing search result from a decoy concatenated databank, decoy data are extracted from the Result File and stored in Proline databases as an independant/unique Search Result as well as target Search result data. These both searches are linked to each other.
- Separated searches
  - The two performed searches are stored in Proline databases and are linked together.

See Search Result to view which information is saved

Identification Summary

An **Identification Summary** (aka ResultSummary) is a set of identified proteins inferred from a subset of the PSM contained in the *Search Result*. The subset of PSM taken into account are the PSM that have been validated a filtering process (example: PSM fulfilling some specified criteria such as score greater than a threshold value).

Content of an Identification Summary

Peptide Set

Protein Set

- Typical Protein
- sameset

- strict subset
- subsumable peptide set

Search Results and Identification Summary content

Search Result

Importing a Result File creates a new Search Result in the database which contains the following informations:

- Search Settings : software name and version, parameters values
- Peak List and Spectrum information: file name, ms-level, precursor moz, …
- Search result data:
  - Proteins
  - Peptide
  - Spectrum
  - 2 type of Matches
    - a Peptide Match is a match between a peptide and a spectrum, with score, fragment matches …
    - a Protein Matches is a match between a peptide and a protein

Mascot result importation

- The peptide matches score correspond to Mascot ion score

OMSSA result importation

- The peptide matches score correspond to the negative common logarithm of the E-value:
  - Score = -log10(E-value)

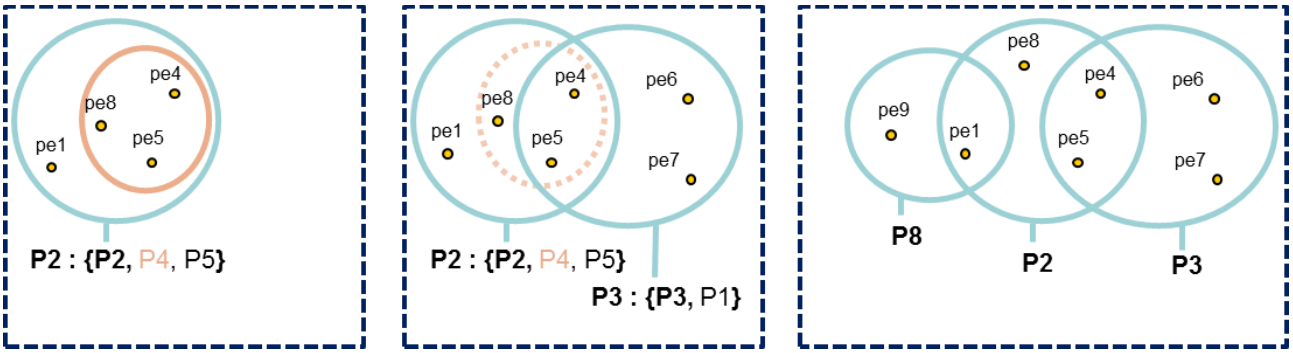
Identification Summary

- Protein Set :
- Peptide Set :

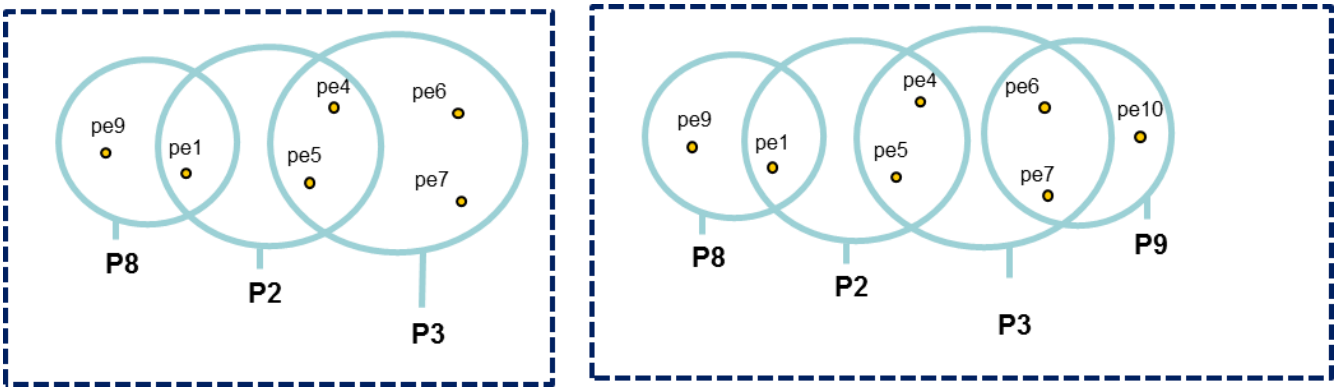
Protein Inference

All peptides identifying a protein are grouped in a `Peptides Set`. A same Peptides Set can identify many proteins, represented by one `Proteins Set`. In this case, one protein of this

Protein Set is chosen to represent the set, it is the `Typical Protein`. If only a sub set of peptides identify a (or some) protein(s), a new Peptide Set is created. This PeptideSet is a subset of the first one, and identified Proteins are `Subset Proteins`.



- In first example, P2 and P5 are identified by the same peptide set {pe1, pe4, pe5, pe8}. P2 was choosen as typical protein. One SubSet composed of {pe4, pe5, pe8} identifies subset protein P4.
- In second example, Another Protein Set represented by P3, shares some peptides with ProteinSet represented by P2. Both ProteinSets have specific peptides.
- Sharing could involve many ProteinSet as shown in example 3.



All Peptides Sets and associated ProteinSets are represented, even if there are no specific peptides. In both cases of above example, no choice is done on which ProteinSet / PeptideSet to keep. These ProteinSets could be filtered after inference (see Protein sets filtering).

Proteins and Proteins sets scoring

There are multiple algorithms than could be use to calculate the Proteins and Protein Sets score. Proteins score are computed during the importation phase while Protein Sets score are comptued during the validation phase.

Protein

Each individual protein match is scored according to all peptide matches associated with this protein, independently of any validation of these peptide matches. Currently, when

- importing Mascot result file : the Mascot standard scoring is used (sum of peptide matches scores)
- importing OMSSA result file :

Protein Set

Each individual protein set is scored according to the validated peptide matches belonging to this protein set (see inference).

Scoring schemes

Mascot Standard Scoring

The score associated to each identified protein (or protein set) is the sum **of the score of all peptide matches** identifying this protein (or protein set). In case of duplicate peptide matches (peptide matched by multiple queries) only the match with the best score is considered.

Mascot MudPIT Scoring

This scoring scheme is also based on the sum of all non-duplicate peptide matches score. However the score for each peptide match is not its absolute value, but the amount that it is above the threshold: the score offset. Therefore, peptide matches with a score below the threshold do not contribute to the protein score. Finally, the average of the thresholds used is added to the score. For each peptide match, the "threshold" is the homology threshold if it exists, otherwise it is the identity threshold. The algorithm below illustrates the MudPIT score computation procedure:

```
Protein score = 0
For each peptide match {
 If there is a homology threshold and ions score > homology threshold {
 Protein score += peptide score - homology threshold
 } else if ions score > identity threshold {
 Protein score += peptide score - identity threshold
 }
}
Protein score += 1 * average of all the subtracted thresholds
```

- if there are no significant peptide matches, the protein score will be 0.
- homology and identity threshold values depend on a given p-value. By default Mascot and Proline compute these thresholds with a p-value of 5%.
- In the case of separated target-decoy searches we obtain two values for each threshold : one for the target search and another one for the decoy search. In order to obtain a single value we apply the following procedure:
    - the homology threshold is the decoy value if it exists else the target value
    - the identity threshold is the mean of target and decoy values.

The benefit of the MudPIT score over the standard score is that it removes many of the junk protein sets, which have a high standard score but no high scoring peptide matches. Indeed, protein sets with a large number of weak peptide matches do not have a good MudPIT score.

Mascot Modified MudPIT Scoring

This scoring scheme, introduced by Proline, is a modified version of the Mascot MudPIT one. The difference with the latter is that it does not take into account the average of the substracted thresholds. This leads to the following scoring procedure:

```
Protein score = 0
For each peptide match {
 If there is a homology threshold and ions score > homology threshold {
 Protein score += peptide score - homology threshold
 } else if ions score > identity threshold {
 Protein score += peptide score - identity threshold
 }
}
```

This score has the same benefits than the MudPIT one. The main difference is that the minimum value of this modified version will be always close to zero while the genuine MudPIT score defines a minimum value which is not constant between the datasets and the proteins (i.e. the average of all the subtracted thresholds).

FDR Estimation

There are several ways to calculate FDR depending on the database search type. In Proline the FDR is calculated at peptide and protein levels using the following rules:

- if the Search has been done on a concatenated Target/Decoy bank, FDR = 2 * nbr DecoyPSM / (nbr TargetPSM + bnr DecoyPSM). Note: when computing PSM FDR, peptide sequences matching a Target Protein and a Decoy Protein is taken into account in both cases.
- if the Search has been done on a separated Target/Decoy bank, FDR = nbr DecoyPSM / nbr TargetPSM.

Validation Algorithm

Once a result file have been imported and a search result created, the validation is performed in 4 mains steps :

1. Peptide Matches filtering and Validation
2. Protein Inference (peptides and proteins grouping)
3. Protein and Proteins Sets scoring
4. Protein Sets Filtering and Validation

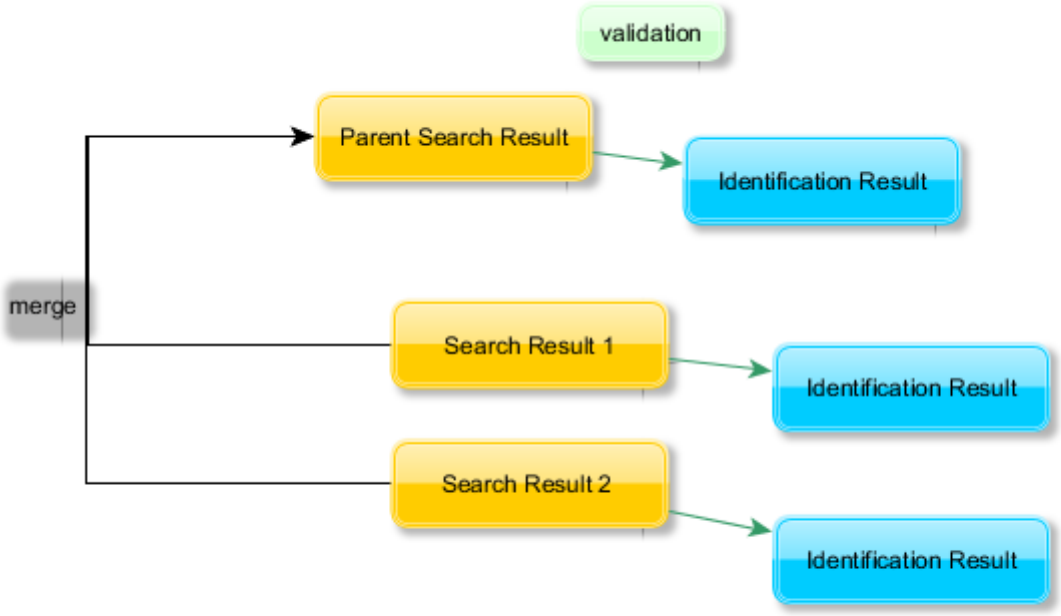
Finally, the Identification Result issued from these steps is stored in the identification database. Different validation of a Search Result can be performed and a new Identification Summary of this Search Result is created for each validation.

Merger Search Results

Merging several Search Results consist in creating a parent `Search Result` which will contain all merged PSMs issued from child `Search Result`. For each identified peptide (in at least one child), a **single** *merged PSM* will be created and filled with the best child attributes (score, missed cleavage, etc). The best child PSM is the PSM with the higher score.

Once a parent `Search Result` is created, the same validation operation as the one accessible for new imported `Search Result` could be done. In this case, the generated `Identification result` is not linked to `Identification Result` associated to child `Search Result`.

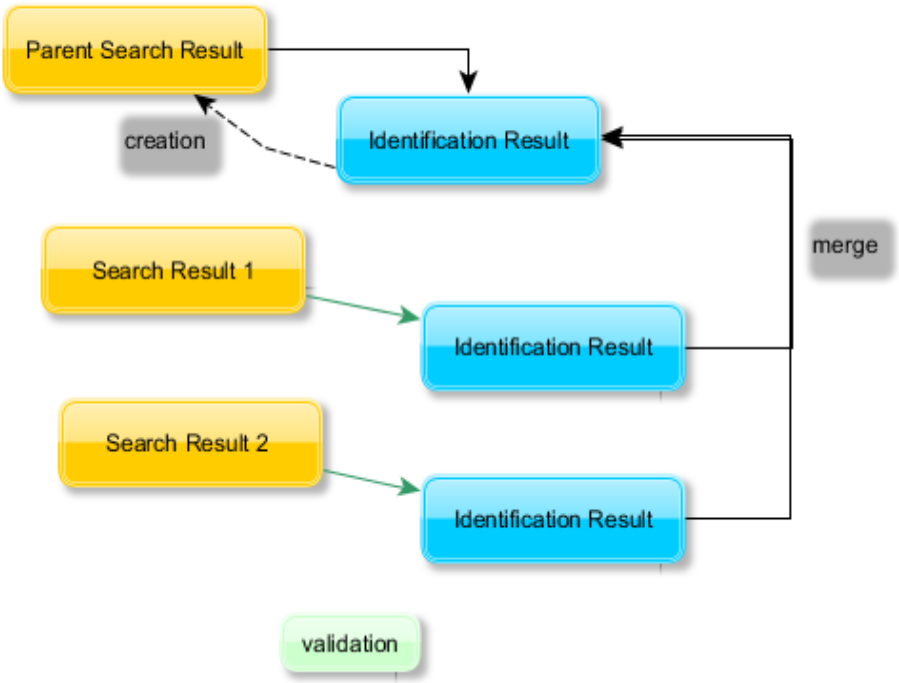
An other merge algorithm could be used : see Merge Identification Results



Merge Identification Results

This merge operation consists in creating a parent `Identification Result` from few child ones. A `Search Result` corresponding to this parent `Identification Result` will be generated.

Concretely, the first step of this merge operation consist in creating merged PSMs for all peptides identified and validated in at least one child `Identification Result`. Protein Inference will then be applied to create the parent `Identification Result`.



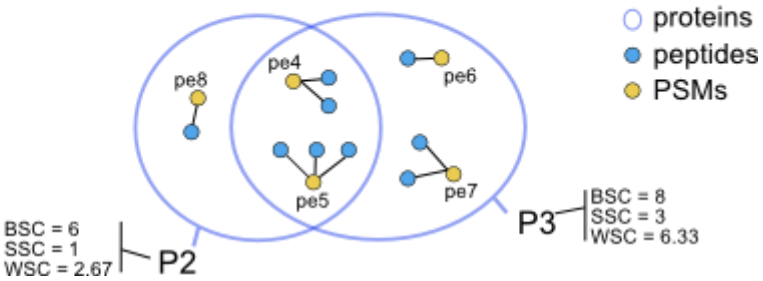
Compare Identification Summaries with Spectral Count

Definition

- The `peptide spectral count` consist in counting the number of spectra which matches the current peptide. Thus, it's equal to the number of pepitde spectrum matches (PSM).
- `Protein basic spectral count` (BSC) is equal to the sum of the `peptide spectral count` for all peptides which identify the protein.
- `Protein specific spectral count` (SSC) is equal to the sum of the `peptide spectral count` for specific peptide only. A specific peptide, is a peptide which does not identify any other protein (or more precisely protein in other protein sets) in the context of the identification summaries .

- `Protein weigthed spectral count` (WSC) is the `Protein specific spectral count` + sharing-weighted spectral count of shared peptide.

SC_{pe4} for P2 = 2 x (1/3) = 0.67
SC_{pe4} for P3 = 2 x (2/3) = 1.33

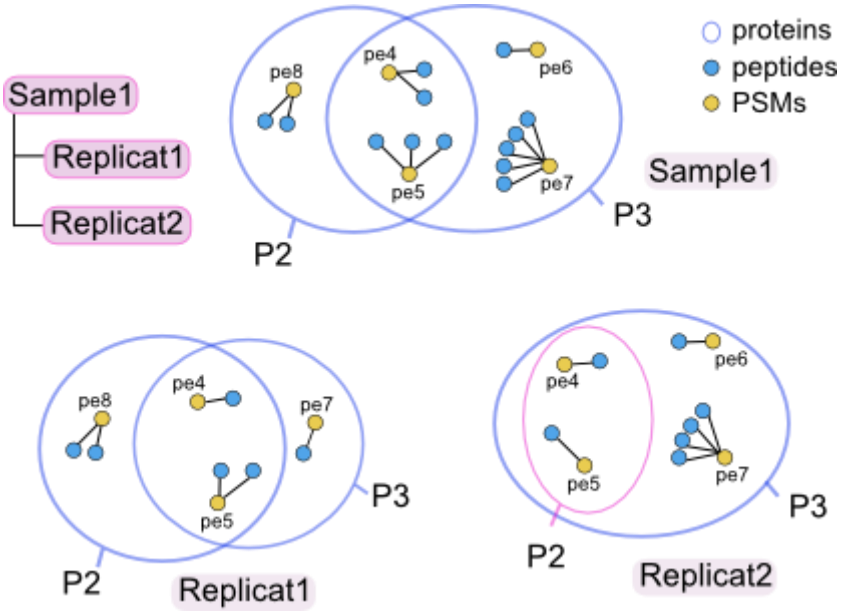


Example calculation of spectral count

Specificity and weigth reference

The peptide specificity and the spectral count weight could be defined in the context of the `Identification Summary` where the spectral count is calculated as shown in previous schema. It could also be done using another `Identification Summary` as reference, like using the common parent `Identification Summary`. This allow to consider only identified and validated protein in the merge context.

If we consider the following case, where *Sample1* `Identification Summary` is the merge of *Replicat1* and *Replicat2*.



If the spectral count calculation is done at each child level, aligning protein sets identified in parent to protein sets in child, we get the following result :

Sample1 ProteinSets	**Replicat1**	**Replicat2**

	Ref Prot.	BSC	SSC	WSC	Ref Prot.	BSC	SSC	WSC	
P2	P2	5	2	3.5	P3	7	7	7	
P3	P3	4	1	2.5	P3	7	7	7	

We can see that when different parent protein sets are seen as one protein set in a child, the spectral count is biased. **This calculation was not retain!**

Now, if we align on child protein rather than protein sets, we get the following result:

Sample1 ProteinSets	**Replicat1**				**Replicat2**				
	Ref Prot.	BSC	SSC	WSC	Ref Prot.	BSC	SSC	WSC	
P2	P2	5	2	3.5	P2	2	0	0	
P3	P3	4	1	2.5	P3	7	7	7	

Again, when considering specificity at protein set level, the result of spectral count in Replicat2 is not representative, as it has a null SSC and WSC. **This calculation was not retain!**

A way to make some correction is to define the specificity of the peptide and their weight at the parent level, and apply it at the child level. Therefore, specific peptides for P2 are pe8 and for P3 it is pe6 and pe7. For peptide weight, if we consider pe4 for example, it will be define as follow:

- Weight pe4 for P2 = 1/3 ⇒ P2 has 1 specific peptide for a total of 3 (if we consider P2 and P3 which are proteins identified by pe4)
- Weight pe4 for P3 = 2/3 ⇒ P3 has 2 specific peptide for a total of 3 …

The spectral count result will thus be :

Sample1 ProteinSets	**Replicat1**				**Replicat2**				
	Ref Prot.	BSC	SSC	WSC	Ref Prot.	BSC	SSC	WSC	
P2	P2	5	2	3	P2	2	0	1	
P3	P3	4	1	3	P3	7	5	6.34	

In Proline

Actually, spectral count is calculated for a set of hierarchy related `Identification Summaries`. In other words, this means that `Identification Summaries` should have a common parent. The list of protein to compare or to considere is created at the parent level as the peptide weigth for spectral count (see previous chapter)

Firstly, the `peptide spectral count` is calculated using following rules

- Equal to Peptide Spectrum Matches Count if Identification Summaries is a leaf (not issued from a merge)
- Sum of child `peptide spectral count` in case of identification Summaries merge
- Sum of validated child `peptide spectral count` in case of Search Result merge. Validated child PSMs are PSMs which satisfy validation applied to parent `Identification Summaries`.

Once, `peptide spectral count` is known for each peptide, protein spectral count is calculated using followig rules

- Protein BSC = sum of `peptide spectral count`
- Protein SSC = sum of `peptide spectral count` for specific peptide only
- Protein WSC = SSC + weighted `peptide spectral count` for shared peptides. The weight of a peptide for a given protein (P1) = peptide SC x (number of specific peptides of P1 /number of peptides specific peptides of all protein identified by the peptide). See explanation in previous chapter

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Quantitation: principles

This section will describe in details the quantitation principles and concepts.

- LC-MS quantification: Different strategies for quantitative analysis
- LC-MS quantification workflows : Workflow and implementation in Proline
- mzDB-processing : Extracting peptidic signals from a file converted into the mzDB format
- Label-free LC-MS quantitation workflow : Label Free specific workflow

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Quantitation: configuration

The first quantitation step as well as the advanced quantitation (see Quantitation : principles) have some parameters that could be modified by the user.

- Label-free LC-MS quantitation configuration
- Advanced Quantitation (Profilizer) configuration

LC-MS quantification

Different strategies for quantitative analysis

Although 2D-gel analysis has been a pioneer method in this field, it has been gradually replaced by nanoLC-MS/MS analysis allowing nowadays to quantify a larger number of proteins and allowing their identification. Quantification is made on thousands of species and requires new and adapted algorithms for the processing of complex data. Two major strategies are available to perform nanoLC-MS/MS relative quantification: strategies based on isotopic-labeling of peptides or proteins in one of the compared conditions, and label-

free based strategies that can be analyzed in different ways. There are usually three types of LC-MS/MS data analyses (cf. figure 1):

- Extraction of a couple of MS signals detected within a single analysis when using a isotopic-labeling strategy
- Counting and comparing the number of fragmentation spectra (MS/MS) of peptides from a given protein detected in parallel analysis ("Label-free quantitation based on spectral-counting"),
- Extraction, alignment and comparison of the MS signal intensities from the same peptide detected in parallel analysis ("Label-free quantitation based on LC-MS signal extraction").

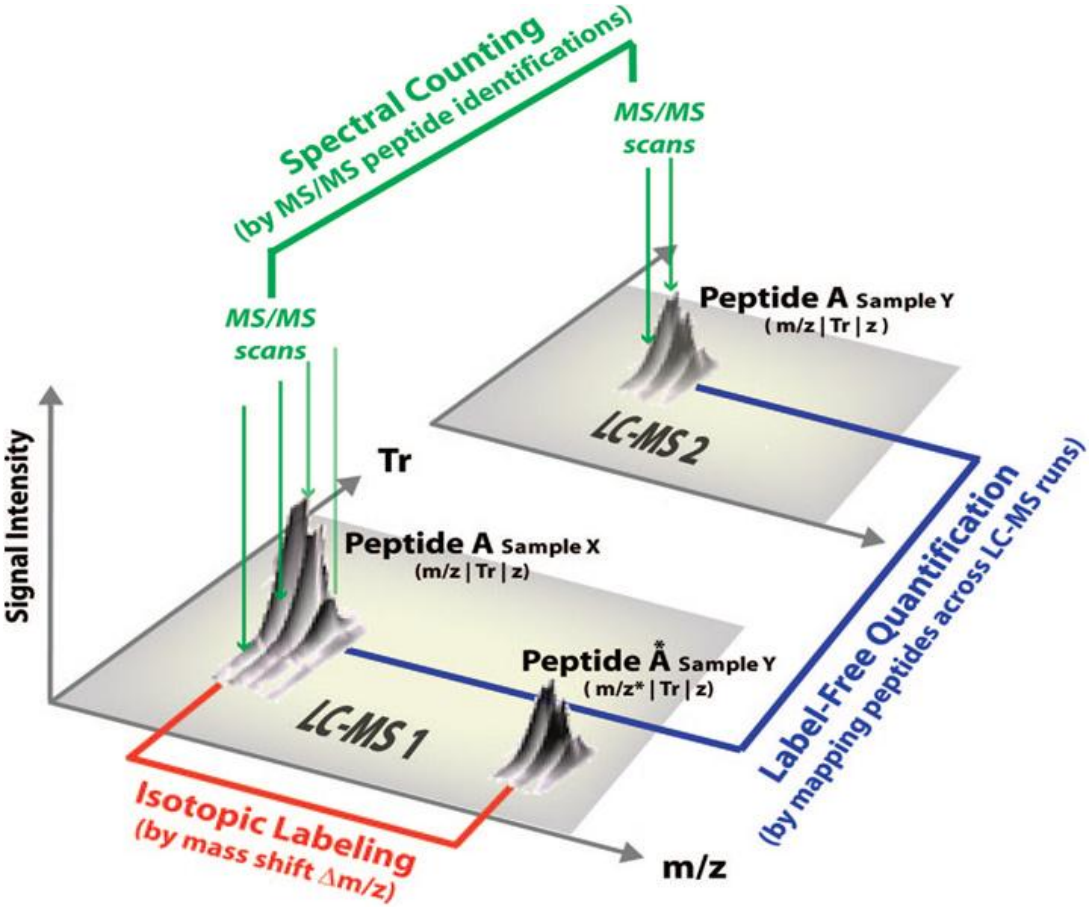

Figure 1: Main view of different approaches of LC-MS/MS quantitative analysis. (Mueller, Brusniak et al. 2008)

At first nanoLC-MS/MS quantitative analysis has been made using isotopic-labeling strategies. Labelling molecules facilitates the relative quantification of two conditions in the same nanoLC-MS/MS run. According to the theory of stable isotope dilution, a isotopically-labelled-peptide is chemically identical to its unlabeled counterpart. Therefore both peptides behave identically during chromatographic separation as well as mass spectrometric analysis (from ionization to detection). As it is possible to measure the difference in mass for the labeled and unlabeled peptide with mass spectrometry, the quantification can be done by integrating and comparing their corresponding signal intensities (cf. figure below).

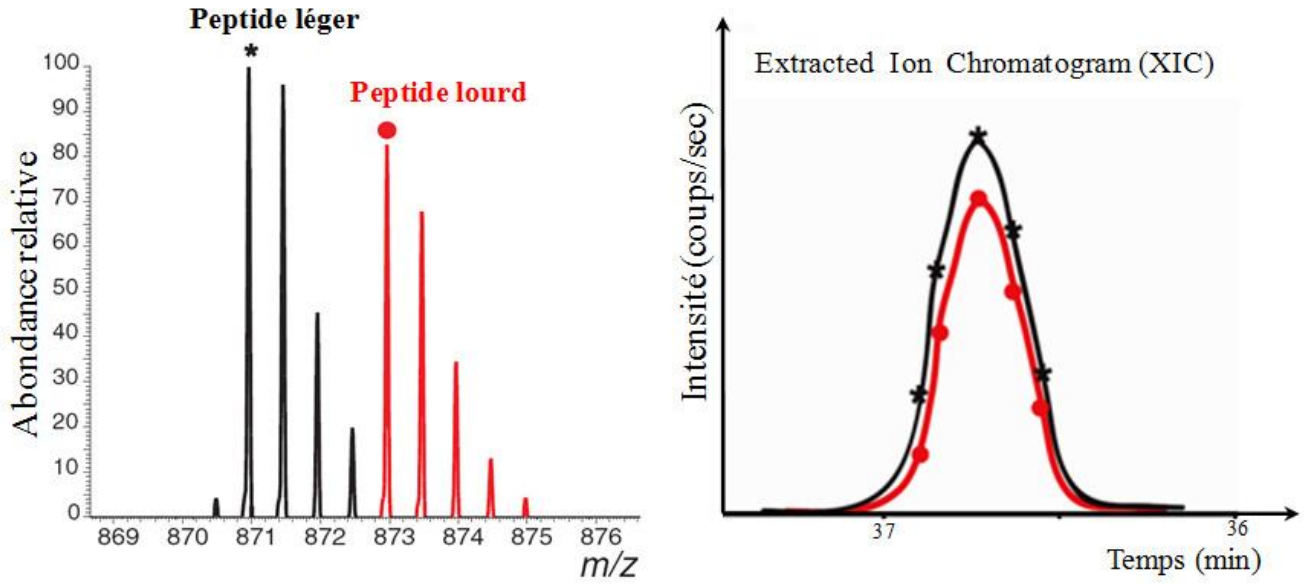

Figure 2: Extraction of quantitative data from a mass spectrum. On the left the visualization of the isotopic profile for each peptide, labeled (red) and unlabelled (black). On the right, the chromatographic peak reconstruction by extracting the signal of the peptide throughtout the duration of the analysis. The integration of this peak gives a proportional value to the abundance of the peptide. Here, the measurement of the areas shows that the abundance of the labelled peptide is 85% that of the unlabelled one.

Isotopic labeling strategies are very efficient but limited by the maximum number of samples that can be compared (eight samples at most for an iTRAQ 8plex labeling), the cost or the constraint due to the introduction of the label. The development of high-resolution instruments, such as the LTQ-Orbitrap, has enabled the development of label-free quantification methods. This methodology is easy to implement as it is no longer necessary to modify the samples, it allows an accurate quantification of the proteins within a complex mixture, and it considerably reduces the cost of the analysis. An LC-MS/MS acquisition can be seen as a map made of all the MS spectra generated by the instrument. This LC-MS map corresponds to a three-dimensional space: elution time (x), m/z (y) and measured intensity (z).

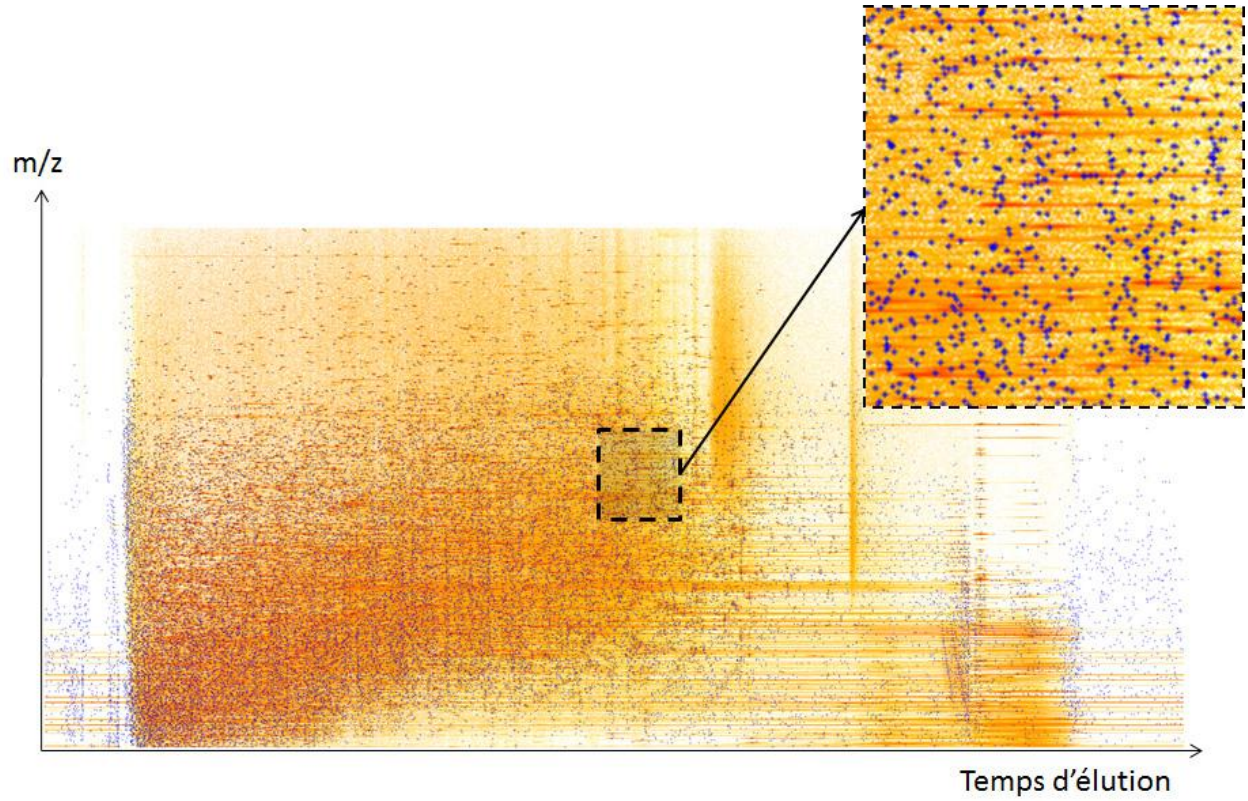

Figure 3: image generated using MsInspect representing an LC-MS map. The dashed square up-right is a zoomed view of the map and gives an idea of the data's complexity. The blue points correspond to the monoisotopic mass of the peptide ions.

Analyzing MS data can be done in several ways:

- Un-supervised approach: it consists of detecting peptide signals from a LC-MS map (cf. figure 3 below). The detection is done by first using peak picking algorithms, then grouping together the peaks that correspond to a same peptide, at the same time on the m/z scale (different isotopes of an isotopic profile and different charge states of a peptide) and on the elution time scale (detected isotopic profiles of the peptide on different consecutive MS spectra all along its chromatographic elution). This process depends on the comparison of experimental data and theoretical known models of isotopic distribution and peptide chromatographic elution. The purpose of this analysis is to find a list of features corresponding to all the signals for a single peptide ion with their corresponding coordinates. The identification of these peptides can be done from the MS/MS spectra matching these features, or using a targeted approach in a second acquisition, or with a database of a set of previously identified peptides containing information such as the peptide sequence, mass and elution time. This third method is called "Accurate Mass and Time Tags" or AMT (Smith, Anderson et al. 2002).
- Supervised approach: the coordinates (x, y) of the peptidic signals to extract are known (or predicted). In an LC-MS experiment, the MS signal intensity of an peptide eluting from the chromatographic column can be monitored (cf. figure 4). The area under the curve of the chromatographic peak is the extracted ion current (XIC, also called extracted ion chromatogram) and it is proportional to the peptide's abundance in the sample. Indeed it has been proved that the XIC is linearly dependant of the quantity of the peptide (Ong and Mann 2005). Therefore

the signal analysis consists of extracting the intensity of the signal at a specific coordinate on the LC-MS map and giving the corresponding XIC.

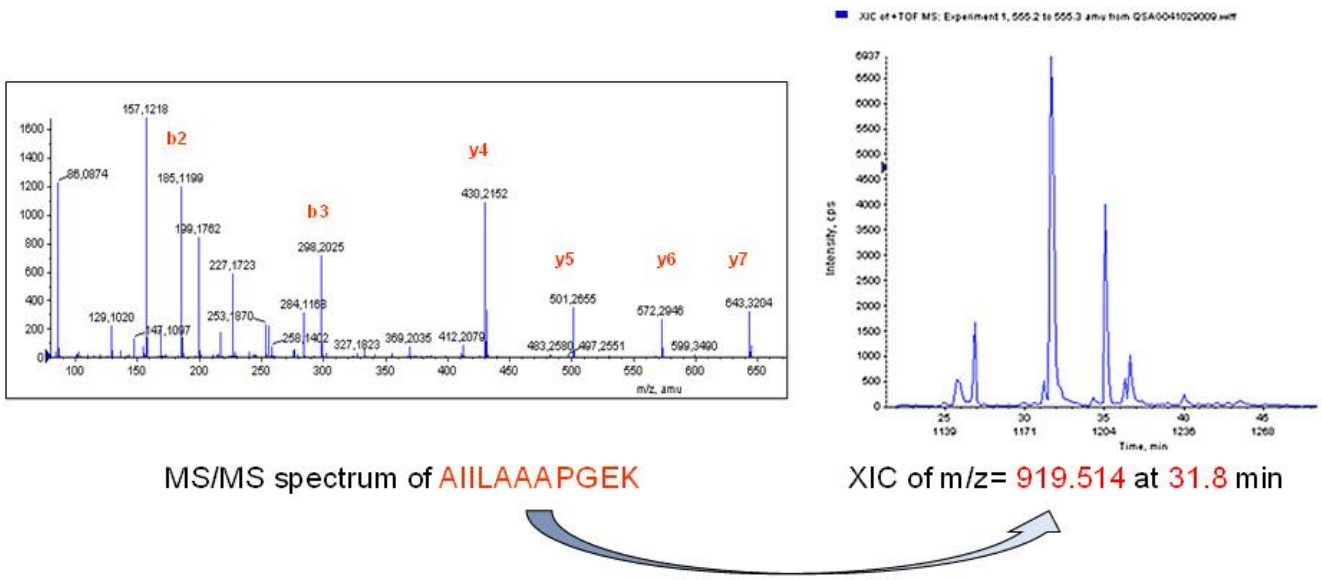

Figure 4: Extraction of the MS signal of a peptide previously identified using a search engine

The first approach is more exhaustive than the latter as it can find quantitative information on peptides that may not have been fragmented by the mass spectrometer. About the second approach, we can only assume that knowing the peptide's exact monoisotopic mass should reduce the probability of making mistakes in the quantification, but no study to our knowledge has proved it so far. In a comparative quantitation analysis, both approaches require the matching of the extracted signals (cf. figure 5). To do this, the LC-MS Maps have to be previously aligned in order to correct the variability coming from the peptide's chromatographic elution. Indeed the difference for the elution time of a given peptide in two LC-MS analysis may reach tens of seconds. Even if a peptide mass can be precisely measured, it is still possible that peptides with very close m/z elute at the same time frame. Figure 3 shows how important the density of the measures is. Therefore, comparing LC-MS maps without aligning their time scale would generate many matching errors.

Figure 5: Matching of the detected peptides on several LC-MS maps

Different algorithms have been developed to correct the time scale and are usually optimized for a given approach. Supervised method benefits of the knowledge of the peptide identification and thus will be able to align maps with a low error rate. More data processing will be needed to obtain quality quantification results. Read the "LC-MS quantitation workflows" documentation to get more information about LC-MS quantification algorithms in Proline.

LC-MS quantification workflows

LC-MS quantification algorithms implemented in Proline are based on the prototype software Prosper developed by IPBS. There are a large number of LC-MS map generation tools. However there was no software solution, until Prosper, able to read and compare data generated by different tools. It was thus a tedious work to evaluate the relative performance of the available peak picking solutions. Proline overcomes this problem by implementing the Prosper's parsers in order to bring a high level of flexibility in the workflows of LC-MS data analysis. Users can use data coming from different peak picking software, and all the rest of the data processing (alignment, normalization, comparison) can be done with Proline's integrated tools. Proline also has its own feature detection algorithms.

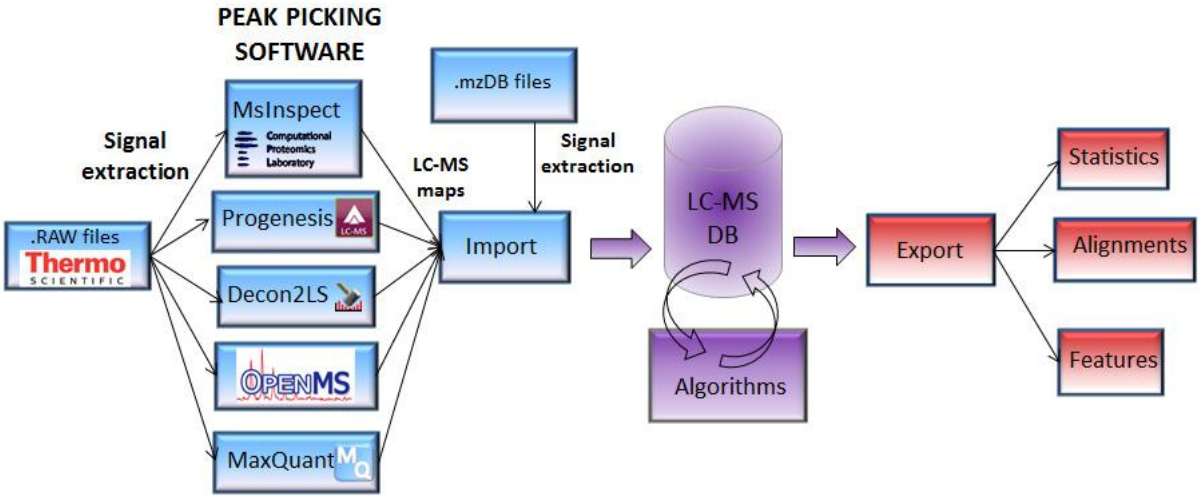

Figure 1: Overview of the LC-MS quantification workflow. LC-MS maps from different sources can be imported in the LCMSdb. Once loaded, these maps will be treated by several algorithms for data processing and comparison. The result of the analysis can be exported into different file formats.

Analytic workflows have been developed for each quantitative analysis strategies.

- Label-free LC-MS quantitation workflow
- Isotopic labeling LC-MS quantitation workflow

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mzDB-processing

Purpose

Extracting peptidic signals (called “features”) from a file converted into the mzDB format.

Feature extraction algorithms

The FeatureExtractor algorithm is composed of four different extraction strategies:

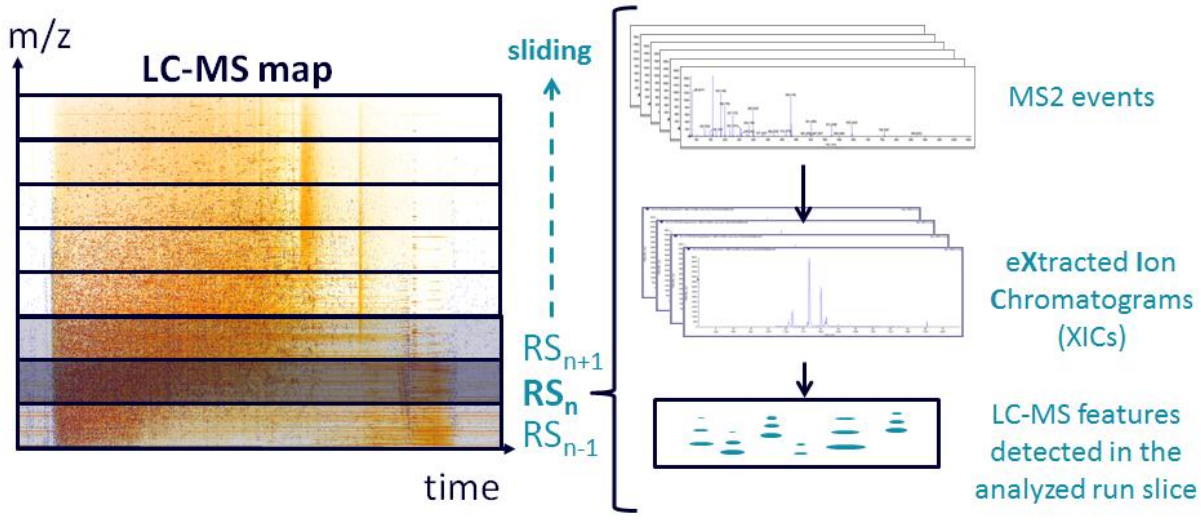
- UnsupervisedFeatureExtractor (NYI)
- MS2DrivenFeatureExtractor
- PredictedTimeFeatureExtractor
- PredictedMzFeatureExtractor (NYI)

The selection of the strategy depends on the `PutativeFeatures` parameters. Details on these different implementations are given in the following sections.

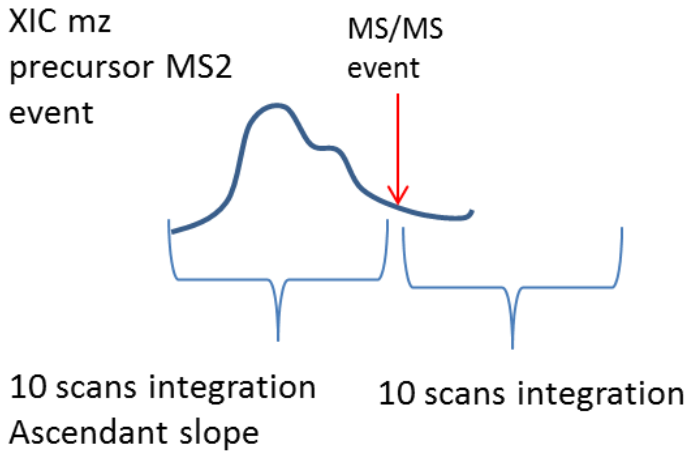
MS2 driven algorithm

This is the main peptide signals extraction algorithm. Every MS/MS event triggered by the spectrometer corresponds to one or more peptidic signal. Each event provides a set of information about the targeted precursor ion: the m/z ratio (assuming it is monoisotopic), the moment when the MS/MS has been triggered (usually not the maximum of the elution peak) and the charge state of the ion. The first and second information can be considered as close coordinates for the peptide signal on the LC-MS map. The charge state (z) can provide additional information to simplify the extraction of different isotopes of the features which are approximately separated by 1/z. For each MS/MS event:

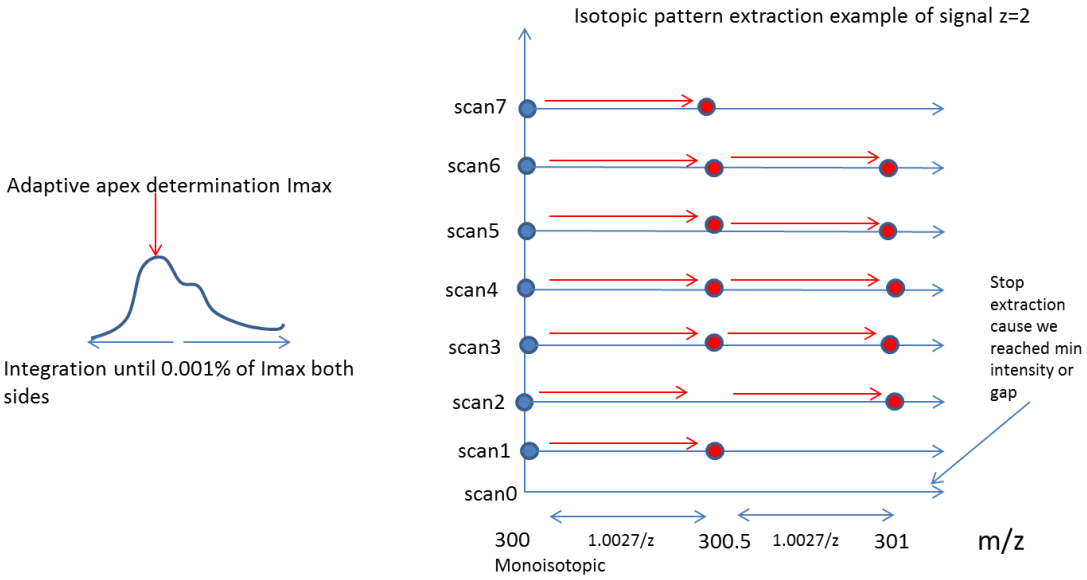
1. The runSlice containing the precursor m/z of the MS/MS event is retrieved (default window is 5 Da, more details in the mzDB documentation), as well as the following runSlice, in order to load into memory everything about the peptidic signal including the isotopes. The XIC for the MS/MS precursor mass can be then easily accessed with a user defined mass precision (default is 5ppm).



2. The apex of the elution peak of the monoisotopic mass of the peptide does not exactly fit to the moment the MS/MS was triggered. Knowing that, the signal on the XIC is integrated on both sides of the moment the MS/MS was triggered (default value is 10 scans) to determine the ascendant slope and in order to find the apex. The integration of the signal is done by summing the intensities of n isotopes, n being a user-defined value (default value is 3, including the monoisotopic peak).



3. For each isotopic profile, the intensities are extracted allowing gaps (default value is 1) until a minimal intensity is reached. This minimal intensity is defined as a ratio of the detected apex intensity (default value is 0.001%). Only one extraction is done per spectrum, hence reducing the extraction time (theoretically).



4. The peak is detected on the extracted signal corresponding to the isotope signal with the highest relative intensity predicted by the averagine (most of the time it corresponds to the monoisotopic peak, in conventional conditions such as trypsic digestion). The limits of this peak are used to tune all the limits of the isotopes (elution peaks). To do so, two different algorithms are being tested:
    1. "Basic" algorithm: applying a Savitsky-Golay smoothing then looking for the local highest point.
    2. Wavelet-based algorithm: using multiple wavelet transformed curves to determine the position of the peaks
5. The last step consists in extracting the peptide signals containing a strong overlap with the previously extracted signal (especially with the first two isotopes).

The extraction of all the signals corresponding to MS/MS events is made in a single iteration on all the runSlices of a mzDB file. Also all the peptide signals which mass are contained in the runSlice are detected simultaneously.

PredictedTimeExtractor algorithm

This algorithm is used for cross-assignment, when a peptidic signal is detected in a file but does not have an equivalent signal in another (frequently in DDA). In this case, the algorithm will try to extract some signal from the file where the signal has not been found. The aim of this algorithm is to reduce the number of missing values.

1. Extracting a 4-minutes XIC (user-defined value) around:
    1. the time predicted by the alignment
    2. the ratio m/z of the isotope with the highest intensity predicted by the averagine (which is estimated from the mean value of the m/z of the observed signals in other conditions)
2. Peaks are detected with the wavelet based algorithm (usually better for a signal made of hundreds of peaks) and limits of time are determined. The isotopic profiles are extracted for each spectrum using the method as in the MS2Driven algorithm. Many peptide signals can be detected and need to be filtered in order to find the best match with the signals in other conditions.
3. To do so, we verify beforehand that :
    1. The chromatographic elution peaks of the monisotopic mass are really corresponding to monoisotopic masses: i.e., if no elution peak P is present before the considered monoisotopic mass M that has a difference of mass equal to 1.0027/z (z being the charge of M), having a distance apex-to-apex (P vs. M) lower than a user-defined threshold of number of cycles (default value is 5), a Pearson correlation higher than a user-defined threshold (default value is 0.7) and finally a P/M area ratio agreeing with the predicted value for P using averagine.
    2. If needed a filter of the duration of a peptide signal (which is usually peptide-specific)
    3. Considering the signals close to each other in time (elution time at the apex vs. predicted time)
    4. Consider the signals close to each other in m/z ratio

Label-free LC-MS quantitation workflow

Analyzing Label-free LC-MS data requires a series of algorithms presented below.

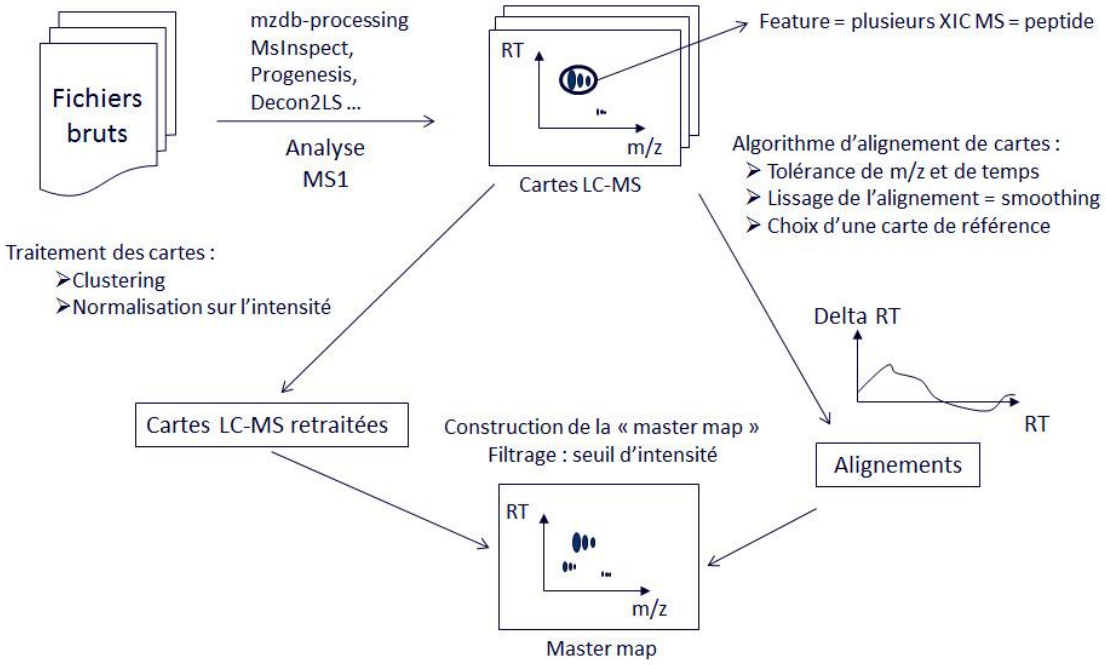


Figure 1 : overview of the differents stages of label-free LC-MS data processing

1. Generation of the LC-MS maps

LC-MS maps can be imported from files generated by others peak picking LC-MS tools, or directly created through Proline with its own feature extraction algorithms.

2. Feature clustering

Maps generated with peak picking algorithms cannot be 100% reliable and often contain redundant signals, corresponding to the same compound. Furthermore, modified peptides having the same sequence can have different PTM polymorphisms that can give different MS signals with the same m/z ratio but having slightly different retention times. Comparing LC-MS maps with such cases is a problem as it may lead to an inversion of feature matches between maps. Creating feature clusters is a way to avoid this issue. This operation is called “Clustering” (cf. figure 2).

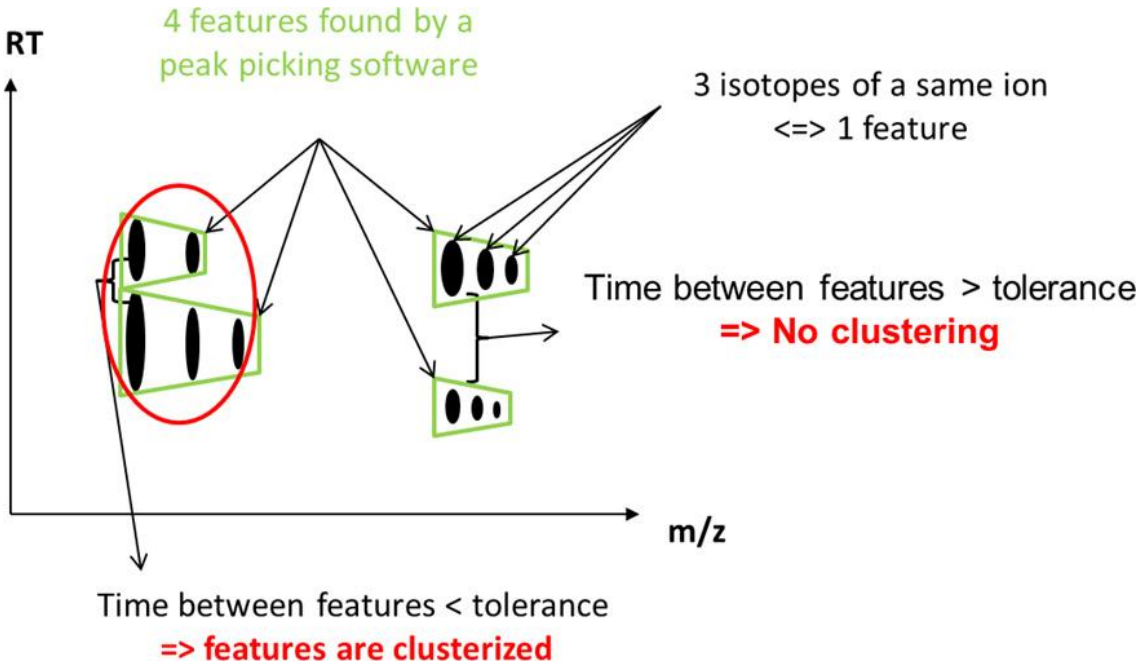


Figure 2 : grouping features into cluster. All features with the same charge state, close m/z ratio and retention times are grouped in a single cluster. The other features are stored without clustering.

The processing consists of grouping, in a given LC-MS map, the features with the same charge state, close in retention time and m/z ratio(Default tolerance is respectively 15 seconds and 10 ppm). Some metrics are calculated for each cluster (equivalent as those used for the features) :

- *Cluster m/z* is the median of the m/z of all features in the cluster
- *Cluster RT* is (2 calculation options):
  - *Median*: median of all the retention times of the features in the cluster
  - *Most intense*: retention time of the most intense feature
- *Cluster intensity* is:
  - *Sum*: sum of the intentisties of all the features in the cluster
  - *Most intense*: intensity of the most intense feature
- *Cluster charge* state is the charge state of every feature in the cluster
- *Number of MS1 in cluster* is the sum of the MS1 signal of all features in the cluster
- *Number of MS2 in cluster* is the sum of the MS2 signal of all features in the cluster
- *Cluster first scan* is the first scan of all the features in the cluster
- *Cluster last scan* is the last scan of all the features in the cluster

The resulting Maps are “cleaner” at the end of the algorithm, thus reducing ambiguities for map alignment and comparison. Quantitative data extracted from these maps will be processes in the following steps. It is necessary to eliminate the ambiguities found by the clustering step. To do so, it is possible to rely on the information given by the search engine on each identified peptide. If some ambiguities remain, the end user must be aware of them and be able to either manually handle them or either exclude them from the analysis.

NB: do not mix up clustering and deconvolution which consists in grouping all the charge states detected for a single molecule.

3. LC-MS map alignment

Feature matching

Because chromatographic separation is not completely reproducible, LC-MS maps must be aligned before being compared. The first step of the alignment algorithm is to randomly pick a reference map and then compare every other map to it. On each comparison the algorithm will determine all possible matches between detected features, considering time and mass windows (the default values are respectively 600 seconds and 10 ppm). Only landmarks involving unambiguous links between the maps (only one feature on each map) are kept (cf. figure 3).

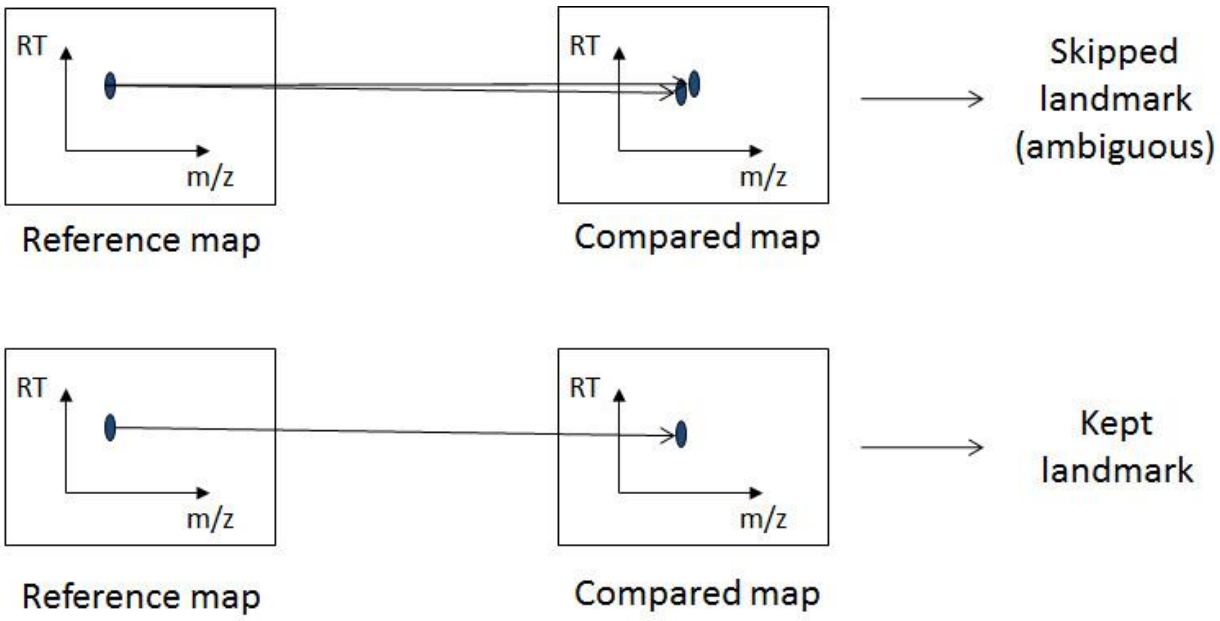


Figure 3 : Matching features with the reference map respecting a mass (10ppm) and time tolerance (600s)

The result of this alignment algorithm can be represented with a scatter plot (cf. figure 5).

Selection of the reference map

The algorithm completes this alignment process several times with randomly chosen reference maps. Then it sums the absolute values of the distance between each map to an average map (cf. figure 4). The map with the lowest sum is the closest to the other maps and will be considered as the final reference map from this point.

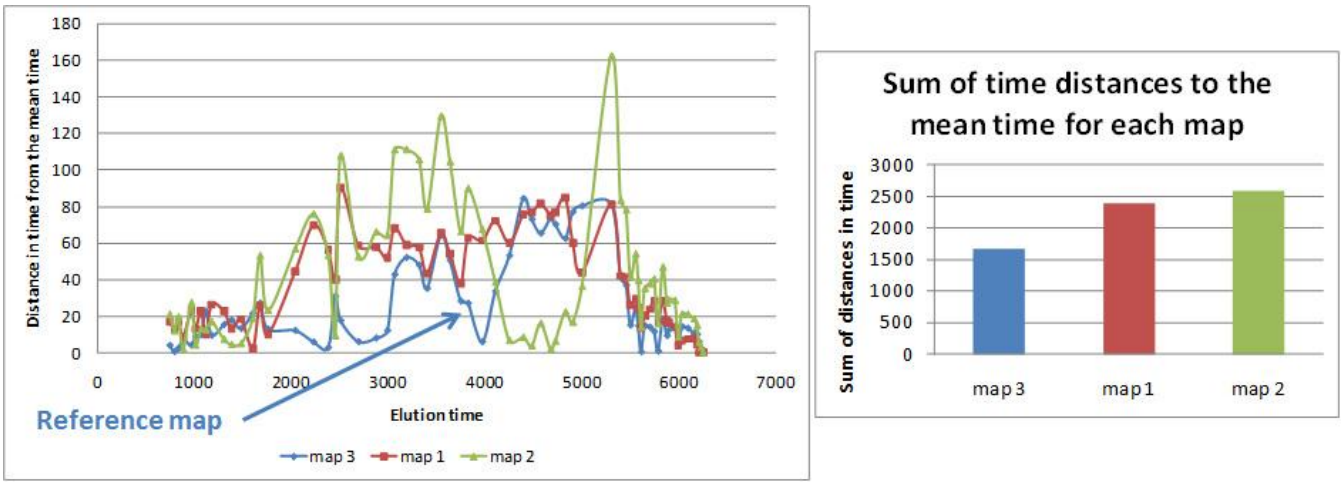


Figure 4 : Selection of the reference map. The chart on the left shows the time distances between each map and the average map obtained by multiple alignments. The chart on the right summarizes the integration of each curve in the chart on the left. The map closest to the average map is selected as the reference map.

Two algorithms have been implemented to make this selection.

Exhaustive algorithm

This algorithm considers every possible couple between maps:

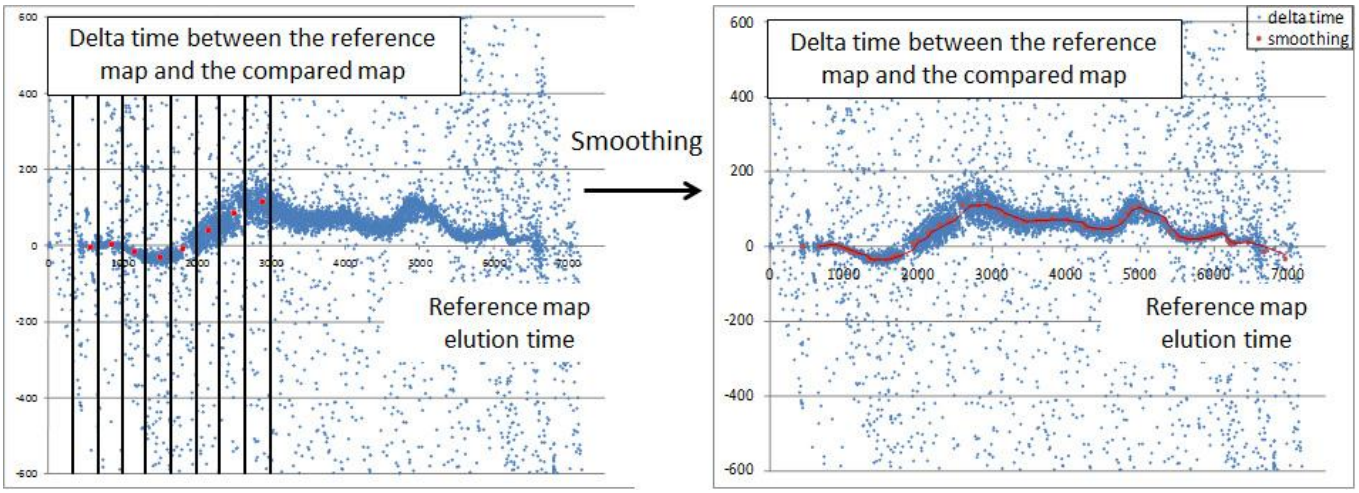
1. For each map, compute the distance in time to all the other maps (Sum of the distances in seconds)
2. The reference map is the one with the lowest distance

Iterative algorithm

1. Randomly select a reference map
2. Align this map with all the other maps
3. Compute the distance in time to all the other maps
4. The new reference map is the one with the lowest distance
5. Steps 2 to 4 are repeated unless
    1. the reference map remains the same for two consecutive iteration
    2. the maximum number of iteration is reached (default value is 3)

Alignment smoothing

The last thing to do is to find the path going through the regions with the highest density of points in the scatter plot. This step was implemented using a moving median smoothing (cf. figure 5).


Delta time between the reference map and the compared map
Smoothing
Reference map elution time
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smoothing
Delta time between the reference map and the compared map
Reference map elution time

Figure 5 : Alignment smoothing of two maps using a moving median calculation. The scatter plot represents the time variation (in seconds) of multiple landmarks (between the compared map and the reference map) against the observed time (in seconds) in the reference map. A user-defined window is moved along the plot, computing on each step a median time difference (left plot). The smoothed alignment curve is constituted of all the median values (right plot).

4. Creation of the master map

Once the maps have been corrected and aligned, the final step consists of creating a consensus map or master map. It is produced by searching the best match for each feature detected on different maps. The master map can be seen as a representation of all the features detected on the maps, without redundancy. (cf. figure 6).

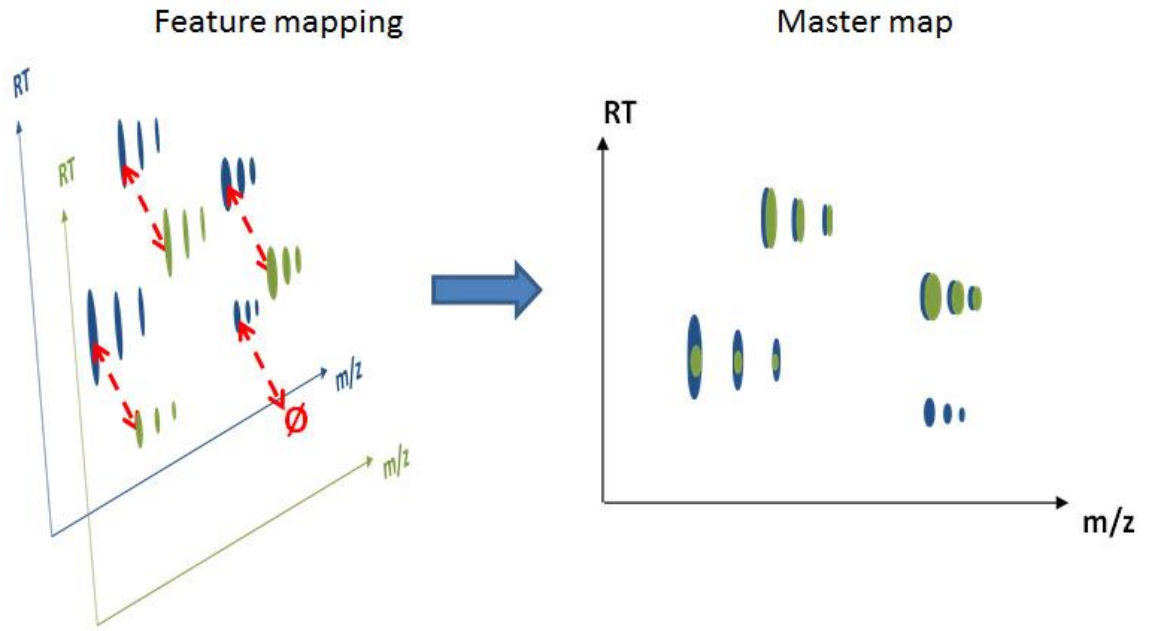


Figure 6 : Creation of the master map by matching the features detected on two LC-MS maps. The elution times used here are the ones corrected by the alignment step. The intensity of a feature can vary from one map to another, it can also happen that a feature only appears in one map.

During the creation of the master map, the algorithm will first consider matches for the most intense features (higher than a given threshold), and then consider the other features only if they match a feature with a high intensity in another map. This is done in order to avoid to include background noise to the master map (cf. figure 7).

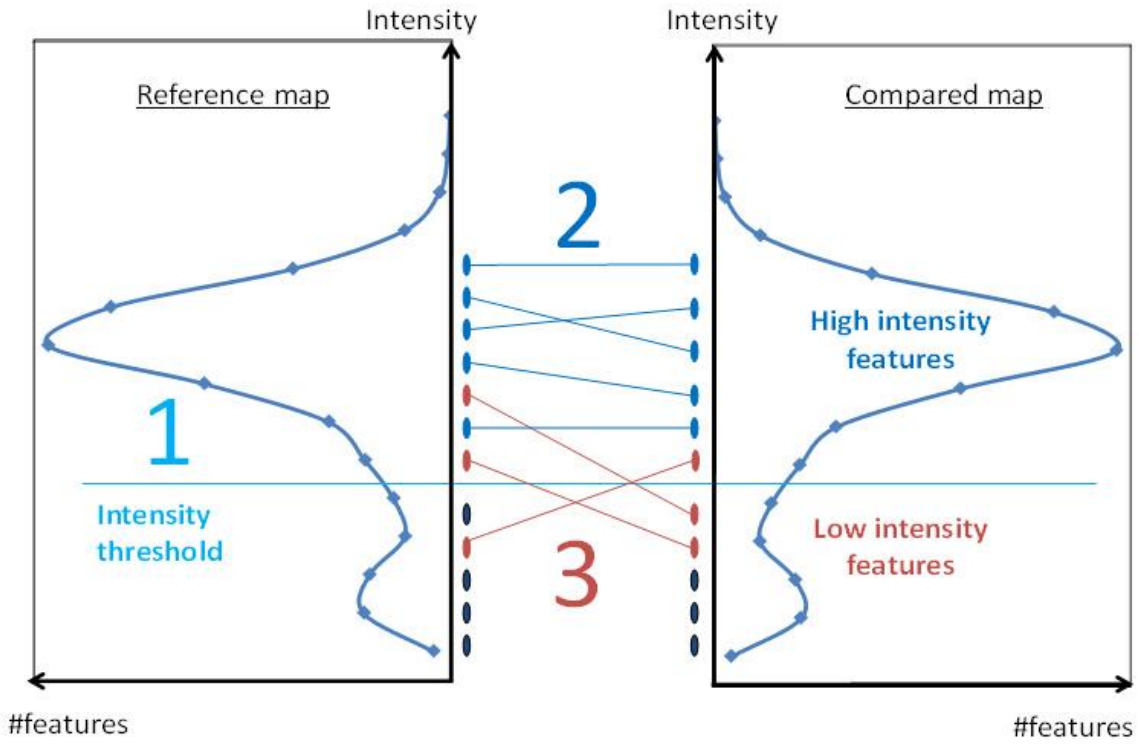


Figure 7 : Distribution of the intensities of the maps considered to build the master map. The construction is done in 3 steps : 1) removing features with a normalized intensity lower than a given threshold 2) matching the most intense features 3) features without matches in at least one map are compared again with the low intensity features, put aside in first step.

5. Solving conflicts

It has been seen that ambiguous features with close m/z and retention times can be grouped into clusters. Other conflicts are also generated during the creation of the master map due to wrong matches. Adding the peptide sequence is the key to solve these conflicts by identifying without ambiguity a feature. Proline has access to the list of all identified and validated PSMs as well as the identifier (id) of each MS/MS spectrum related to an identification. This means that the link between the scan id and the peptide id is known. On the other hand the list of MS/MS events simultaneous to the elution window of each feature is known. For each of these events the corresponding peptide sequences can be retrieved. If only one peptide sequence is found for the master feature, it will be kept as it is. Otherwise the master feature will be cloned in order to have one feature per peptide sequence. During this duplication step the daughter features will be distributed on the new master features according to the identified peptide sequences.

6. Cross assignment

When the master map is created some intensity values could be missing. Proline will read the mzDB files to reduce the number of missing values, using the expected coordinates (m/z – RT) for each missing feature to extract new features. These new extractions are added to copies of the daughters and the master maps. This gives a new master map with a limited number of missing values.

7. Normalizing LC-MS maps

The comparison of LC-MS maps is confronted to another problem which is the variability of the MS signals measured by the instrument. This variability can be technical or biological. Technical variations between MS signals in two analyses can depend on the injected quantity of material, the reproducibility of the instrument configuration and also the software used for the signal processing. The observed systematic biases on the intensity measurements between two successive and similar analysis are mainly due to errors in the total amount of injected material in each case, or the nanoLC-MS system instabilities that can cause variable performances during a series of analysis and thus a different response in MS signal for peptides having the same abundance. Data may not be used if the difference is too important. It is always recommended to do a quality control of the acquisition before considering any computational analysis. However, there are always biases in any analytic measurement but they can usually be fixed by normalizing the signals. Numerous normalization methods have been developed, each of them using a different mathematical approach (Christin, Bischoff et al. 2011). Methods are usually split in two categories, linear and non-linear calculation methods, and it has been demonstrated that linear methods can fix most of the biases (Callister, Barry et al. 2006). Three different linear methods have been implemented in Proline by calculating normalization factors as the ratio of the sum of the intensities, as the ratio of the median of the intensities, or as the ratio of the median of the intensities.

Sum of the intensities

How this factor is calculated:

1. For each map, sum the intensities of the features
2. The reference map is the median map
3. The normalization factor of a map = sum of the intensities of the reference map / sum of the intensities of the map

Median of the intensities

How this factor is calculated:

1. For each map, calculate the median of the intensities in the map
2. The reference map is the median map
3. The normalization factor of a map = median of the intensities of the reference map / median of the intensities of the map

Median of ratios

This last strategy has been published in 2006 (Dieterle, Ross et al. 2006) and gives the best results. It consists of calculating the intensity ratios between two maps to be compared then set the normalization factor as the inverse value of the median of these ratios (cf. figure 8). The procedure is the following:

1. For each map in a “map set”, sum the intensities of the features
2. The reference map is the median map
3. For each feature of the master map, ratio = intensity of the feature in the reference map / intensity of the feature for this map
4. Normalization factor = median of these ratios

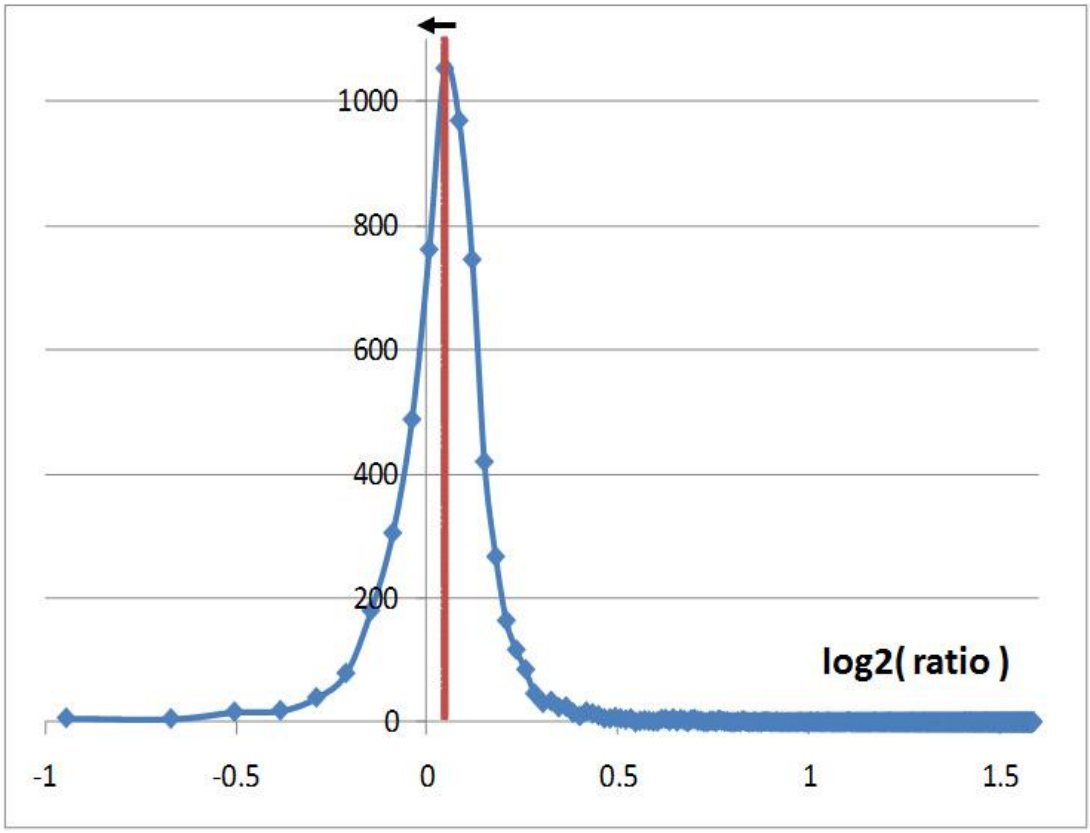


Figure 8 : Distribution of the ratios transformed in log2 and calculated with the intensities of features observed in two LC-MS maps. The red line representing the median is slightly off-centered. The value of the normalization factor is equal to the inverse of this median value. The normalization process will refocus the ratio distribution on 0 which is represented by the black arrow.

Proline makes this normalization process for each match with the reference map and has a normalization factor for each map, independently of the choice of the algorithm. The normalization factor for the reference map is equal to 1.

8. Building a "QuantResultSummary"

Once the master map is normalized, it is stored in the Proline LCMS database and used to create a “QuantResultSummary”. This object links the quantitative data to the identification data validated in Proline. This “QuantResultSummary” is then stored in the Proline MSI database (cf. figure below).

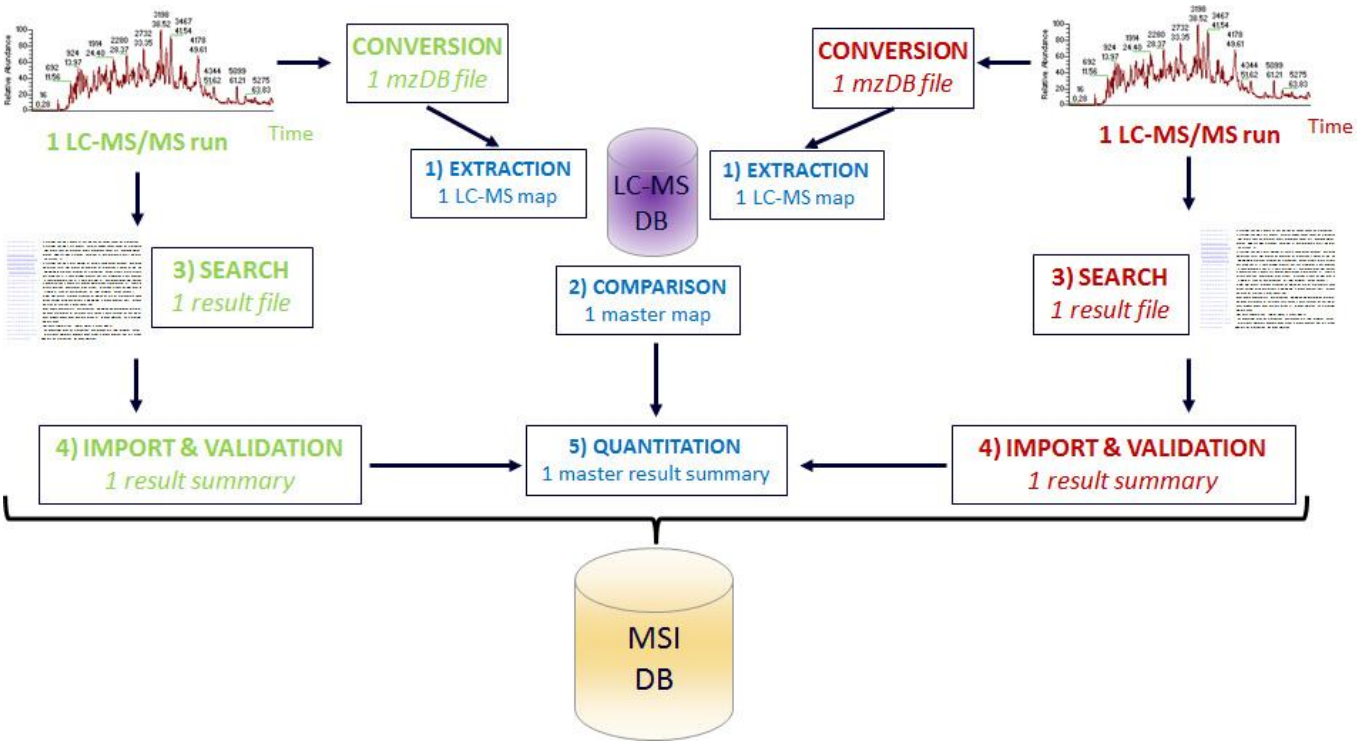


Figure 9 : From raw files to the « QuantResultSummary » object.

Label-free LC-MS quantitation configuration

Here is the description of the parameters that could be modified by the user.

Feature extraction Strategy

Defines the algorithms and methods to used for signal extraction and deistotping.

- Start Extraction from XIC from :
    1. **MS/MS Events**: supervised strategy where each feature extraction is targeted for each acquired MS/MS spectrum.
    2. **Validated Peptides**: same strategy but with a filtering of MS/MS events based on the list of validated peptides.
    3. **Raw MS signal analysis**: unsupervised strategy which tries to detect LC-MS features using a signal recognition algorithm

- Deisotoping mode:
    1. **Unsupervised**: an algorithm combining time correlated isotopes elution peaks. In the final step, this algorithm checks the matching between experimental and theoretical isotopes ratios.
    2. **Identification based**: the charge state of each PSM (Peptide Spectrum Match) is used to combine istotpes signals into an LC-MS feature.

Extraction parameters

Parameters use by signal extraction algorithms

Extraction m/z Tolerance: In supervised algorithms this correpsonds to the error tolerance between the precursor ion m/z and peaks extracted in the mzDB file.
In unsupervised algorithms this corresponds to the the error tolerance between each peak apex and other extracted peaks.

Clustering parameters

Clustering must be applied to the imported LC-MS maps to group features that are close in time and m/z. This step reduces ambiguities and errors that could occur during the feature mapping phase.

- Time tolerance: features that are close in time are grouped. If delta time between two features is lower than time tolerance, features are grouped.
- m/z tolerance: features that are close in m/z are grouped. If delta m/z between two features is lower than m/z tolerance, features are grouped.
- m/z tolerance unit: m/z tolerance can be provided in PPM or Dalton.
- Cluster time computation: you have the choice between 2 computation methods: most intense or median. For most intense method, the cluster time corresponds to the time of the most intense feature composing the cluster. For median method, cluster time is the median of the feature times forming the cluster.
- Cluster intensity computation: you have the choice between 2 computation methods: most intense or sum. For most intense method, the cluster intensity corresponds to the intensity of the most intense feature of features forming the cluster. For sum method, cluster intensity is the sum of the intensities of features composing the cluster.

Alignment Computation

This is an important step in the LCMS process. It consists of aligning maps of the map set to correct the RT values. RT shifts of shared features between the compared maps follow a curve reflecting the fluctuations of the LC separation. The time deviation trend is obtained by computing a moving median using a smoothing algorithm. This trend is then used as a model of the alignment of the compared LC-MS maps. This model provides a basis for the correction of RT values.

- Method : You have the choice between 2 alignment methods
    1. Comprehensive: the comprehensive algorithm computes the distance between maps for each possible couple of maps and selects the map with the lowest sum of distances to be the reference map.
    2. Iterative: for the iterative algorithm, first a reference map is chosen randomly, then each other map is aligned against the reference and the algorithm computes the distance for each couple of maps. The map that has the smallest distance becomes the reference map. The 2 previous steps are re-iterated until either reference map stays the same between two iterations or the maximum number of iterations is reached.

Then all other maps are aligned to this computed reference map and their retention times are corrected.

- Maximum number of iterations: this option is available only for iterative method. This is a stop condition of the iterative algorithm, when the algorithm has reached its maximum number of iterations, it stops.

- m/z tolerance: m/z window used to match features between two compared maps.

- m/z tolerance unit: m/z tolerance can be provided in PPM or Dalton.

- Time tolerance in seconds: time window used to match features between two compared maps.

Alignment smoothing

When alignment is done, a trend can be extracted with a smoothing method permitting the correction of the aligned map retention time.

- Smoothing method: you have the choice between 2 smoothing methods, time window or landmark range.

- Number of landmarks/time interval: if selected smoothing method is landmark range, time of aligned map is corrected using median computed on windows containing a specified number of landmarks. The run is divided in windows of size the specified number of landmarks. You have to provide the number of landmarks by window. The smoothing method is applied considering the number of landmarks present in the window, and computes the median point for this window.

If selected smoothing method is set to time window, time of aligned map is corrected using median in a time window. You have to provide the time interval. This time interval corresponds to the window size in which time median will be computed.

- Minimum number of landmarks in window: this option is only available for time window smoothing method. This allows you to specify the minimum number of landmarks a window must contain to compute a median on it, it is not significant to compute a median on less landmarks.

- Sliding window overlap: overlap is used to compute the step to move the smoothing window forward to calculate a smoothing point for this new smoothing window. Overlap gives the percentage of overlapping between two consecutive windows. For example, if window size is 200 (seconds or landmarks depending on which smoothing method is selected) and overlap is 20%, the step forward = 200*((100-20)/100) = 160 seconds or landmarks, i.e. the smoothing window will be moved forward by a step of 160, so two successive windows will overlap each other by a step of 40 seconds or landmarks corresponding to 20% of 200.

Master map creation

This step consists in creating the “master map” (also called consensus map), this map resulting from the superimposition of all compared maps.

- m/z tolerance: when mapping features from 2 different maps of the map set, delta m/z between features must be lower than the m/z tolerance to be considered as the same feature seen on 2 different maps.

- m/z tolerance unit: the m/z tolerance unit can be provided in PPM or Dalton.

- Time tolerance (seconds): when mapping features from 2 different maps of the map set, delta time between features must be lower than the time tolerance to be considered as the same feature seen on 2 different maps.

- Normalization method: sometimes the ratio distribution is not centered around zero as we could have expected if data were exactly reproducible. Intensity normalization (by applying a mathematical transformation) is thus needed to reduce the impact of experimental artifacts and ensure accurate quantification. Three methods are available:
    1. Median ratio normalization method algorithm: first, compute sum of feature intensities for each map of the map set and sort maps by computed intensities. The map ranking nearest from the median is taken as the reference map. Then for each master map feature, compute ratio as reference map feature intensity / feature intensity for the considered map. The normalization factor corresponds to the median of the computed ratios.
    2. Median normalization method algorithm: first compute median intensity for each map, set the reference map to median map, normalization factor for map M = reference map median intensity / map M median intensity.
    3. Sum normalization method algorithm: first, compute feature intensities sums for each map, set the reference map to the median map, normalization factor for map M = intensities sum of reference map / intensities sum of map M.

- Master feature filter type: a filter can be applied to the map features to keep the best features (above threshold) to build the master map.

Two methods are available to filter features: the filter can be applied directly on intensity values (Intensity method) or it can be a proportion of the map median intensity (Relative intensity method).

- Relative intensity threshold/Intensity threshold: this provides the threshold for one or the other filtering method, depending on which method you have selected. Only features above this threshold will be considered for the master map building process.

- Relative intensity/Intensity method: this option depends on which filtering method you select.

If you choose Relative intensity for master feature filter type, the only possibility you have is percent, so you will remove features which intensities are beyond the relative intensity threshold in percentage of the median intensity. If you choose Intensity for master feature filter type, you also have only one possibility at the moment of the intensity method: basic. Features which intensities are beyond the intensity threshold set will be removed and not considered for the master map building process.

Post-processing of LC-MS quantitative results

This procedure is used to compute ratios of peptide and protein abundances. Several filters can also be set to increase the quality of quantitative results.

Here is the description of the parameters that could be modified by the user.

Peptide filters

- **Use only specific peptides**: if checked, peptides shared between different protein sets will be discarded from the statistical analysis.

- **Discard missed cleaved peptides**: if checked, peptides containing missed cleavages will be discarded from the statistical analysis. It has to be noted that perfect tryptic peptides whose sequence is included in an observed missed cleaved peptide are also discarded if this option is enabled.

- **Discard oxidized peptides**: if checked, peptides containing the Oxidation(M) modification will be discarded from the statistical analysis. It has to be noted that non-modified peptides whose sequence is the same than an observed oxidized peptide are also discarded if this option is enabled.

Peptide and protein common parameters

- Normalization: the normalization factors are computed as the median of the ratios distrubutions between each run and a run of reference. A similar procedure is used for the normalization of LC-MS features.

Aggregation of peptides in proteins

Peptide abundances can be summarized into protein abundances using several mathematical methods:

- sum: for each quantitative channel (raw file) the sum of observed peptides abundances is computed

- mean: for each quantitative channel (raw file) the mean of observed peptides abundances is computed

- mean of TOP3 peptides: same procedure but applied on the 3 most abundant peptides. Peptides are sorted by descending median abundances (computed accross all compared samples for peptide). Then the 3 first peptides are kept.

- median: for each quantitative channel, the median of observed peptides abundances is computed

- median profile: a matrix of peptide abundance ratios is first computed (rows correspond too peptides and columns to quantitative channels). The median of these ratios is then computed for each column. The relative values are then converted back into absolute values using a scaling factor. This factor is computed as the maximum value from the **means of TOP3 peptides** abundances.

- normalized median profile: matrix of peptide abundance ratios is first computed (rows correspond too peptides and columns to quantitative channels). This matrix is then normalized and then summarized using the median method described above. The obtained median abundances are then adjusted by using a scaling factor. This factor is computed as the maximum value from the **means of TOP3 peptides** abundances.

Peptide Matches Filtering

Peptide Matches identified in search result can be filtered using one or multiple predefined filters (describes here after). Only validated peptide matches will be considered for further steps.

Basic Score Filter

All PSMs which score is lower than a given threshold are invalidated.

Pretty Rank Filter

This filtering is performed after having temporarily joined target and decoy PSMs corresponding to the same query (only really needed for separated forward/reverse database searches). Then for each query, PSMs from target and decoy are sorted by their score. A rank (Mascot pretty rank) is computed for each PSM depending on their score position: PSM with almost equal score (difference < 0.1) are assigned the same rank. All PSMs with rank greater than specified one are invalidated.

Minimum Sequence length Filter

PSMs corresponding to short peptide sequences (length lower than the provided one) can be invalidated using this parameter.

Mascot eValue Filter

Allows to filter PSMs by using the Mascot expectation value (e-value) which reflects the difference between the PSM score and the Mascot identity threshold (p=0.05). PSMs having an e-value greater than the specified one are invalidated.

Mascot adjusted eValue Filter

Proline is able to compute an adjusted e-value. It first selects the lowest threshold between the identity and homology ones (p=0.05). Then it computes the e-value using this selected threshold. PSMs having an adjusted e-value greater than the specified one are invalidated.

Mascot p-value on Identity Filter

Given a specific p-value, the Mascot identity threshold is calculated for each query and all peptide matches associated to the query with a score lower than calculated identity threshold are invalidated.
When parsing Mascot result file, the number of PSM candidate for a spectra is saved and could be used to recalculate identity threshold for any p-value.

Mascot p-value on homology Filter

Given a specific p-value, the Mascot homology threshold is inferred for each query and all peptide matches associated to the query with a score lower than calculated homology threshold are invalidated.

Single PSM Per Query Filter

This filter will validate only **one PSM per Query**. To select a PSM, following rules will be applied :

For each query :

- Select PSM with higher score.
- If several PSM with same score :
    - Choose PSM which identify the protein which have the max nmber of valid PSM
    - If still equality
        - Choose the first PSM

For **testing purpose**, it is possible to ask for this filter to be executed after Peptide Matches Validation (see bellow). In this case, the requested FDR in validation step will be modified by this filter. This is just to confirm the need or not of this filter and to validate the way we apply it !

Single PSM Per Rank Filter

This filter will validate only **one PSM per Pretty Rank**. If you choose this filter + a Pretty rank filter you should have the same behaviour than the “Single PSM Per Query Filter”.

In order to choose the PSM, following rules will be applied :

For Pretty Rank of each query :

- If several PSM :
    - Choose PSM which identify the protein which have the max nmber of valid PSM

- If equality
  - Choose the first PSM

This filter is actually in **test** with “Single PSM Per Query Filter”. Finally a decision of with filter and how it is apply will be taken.

Peptide Matches Validation

Specify an expected FDR and tune a specified filter in order to obtain this FDR. See how FDR is calculated

Once previously described pre-filters have been applied, a validation algorithm can be run to control the FDR: given a criteria, the system will estimate the better threshold value in order to reach a specific FDR.

Proline Web installation procedure

Protein Sets Filtering

Specific peptides Filter

Invalid Protein Set that don't have at least x peptides identifying only that protein set. The specificity is considered at the DataSet level.

This filtering go through all Protein Sets from worth score to best score. For each, if the protein set is invalidated, associated peptides properties are updated before going to next protein set. Peptide property is the number of identified protein sets.

Protein Sets Validation

Once pre-filters (see above) have been applied, a validation algorithm can be run to control the FDR. See how FDR is calculated

At the moment, it is only possible to control the FDR by changing the Protein Set Score threshold. Three different protein set scoring functions are available.

Given an expected FDR, the system will try to estimate the best score threshold to reach this FDR. Two validation rules (R1 and R2) corresponding to two different groups of protein sets (see below the detailed procedure) are optimized by the algorithm. Each rule defines the optimum score threshold allowing to obtain the closest FDR to the expected one for the corresponding group of protein sets.

Here is the procedure used for FDR optimization:

- protein sets are segregated in two groups, the ones identified by a single validated peptide (G1) and the ones identified by multiple validated peptides (G2), with potentially multiple identified PSMs per peptide.

- for each of the validation rules, the FDR computation is performed by merging target and decoy protein sets and by sorting them by descending score. The score threshold is then modulated by using successively the score of each protein set of this sorted list. For each new threshold, a new FDR is computed by counting the number of target/decoy protein sets having a score above or equivalent to this value. The procedure stops when there are no more protein sets in the list or when a maximum FDR of 50% is reached. It is has to be noted that the two validation rules are optimized separately:
    - G2 FDR is first optimized leading to the R2 score threshold. The validation status of G2 protein sets is then fixed.
    - final FDR (G1+G2) is then optimized leading to the R1 score threshold. Only the G1 protein sets are here used for the score threshold modulation procedure. However the FDR is computed by taking into account the G2 validated target/decoy protein sets.

The separation of proteins sets in two groups allows to increase the power of discrimination between target and decoy hits. Indeed, the score threshold of the G1 group is often much higher than the G2 one. If we were using a single average threshold, this will reduce the number of G2 validated proteins, leading to a decrease in sensitivity for a same value of FDR. In the future, we will try to implement such a strategy in order to allow the user to make its own comparison.

Search Result and Identification Summary

A **Search Result** (aka ResultSet in the database schema) is the raw interpretation of a given set of MS/MS spectra given by a search engine or a denovo interpretation process. The content of a **Search Result** is one or many peptides matching to the submitted MSMS spectrum (**PSM**), each peptide being issued from protein sequences. The Search Result also contains additional information such as search parameters, used data-bank, etc. A **Search Result** is created when a Result File (Mascot `.dat` file or an OMSSA `.omx`) file is imported in Proline, in the case of a target-decoy search, two **Search Results** are created : one for the target PSM, one for decoy PSM.

An **Identification Summary** (aka ResultSummary) is a set of identified proteins inferred from a subset of the PSM contained in the *Search Result*. The subset of PSM taken into account are the PSM that have been validated a filtering process (example: PSM fulfilling some specified criteria such as score greater than a threshold value).

Content of a Search Result

Importing a Result File creates a new Search Result in the database which contains the following informations:

- Search Settings : software name and version, parameters values
- Peak List and Spectrum information : file name, ms-level, precursor moz, …
- Search result data:
    - Proteins
    - Peptide
    - Spectrum
    - 2 type of Matches
        - a Peptide Match is a match between a peptide and a spectrum, with score, fragment matches …
        - a Protein Matches is a match between a peptide and a protein

Mascot result importation

: todo

The peptide matches score correspond to Mascot ion score

OMSSA result importation

: todo

The peptide matches score correspond to the negative common logarithm of the E-value:

- Score = -log10(E-value)

Content of an Identification Summary

- Protein Set :
- Peptide Set :

Identification Summary Export

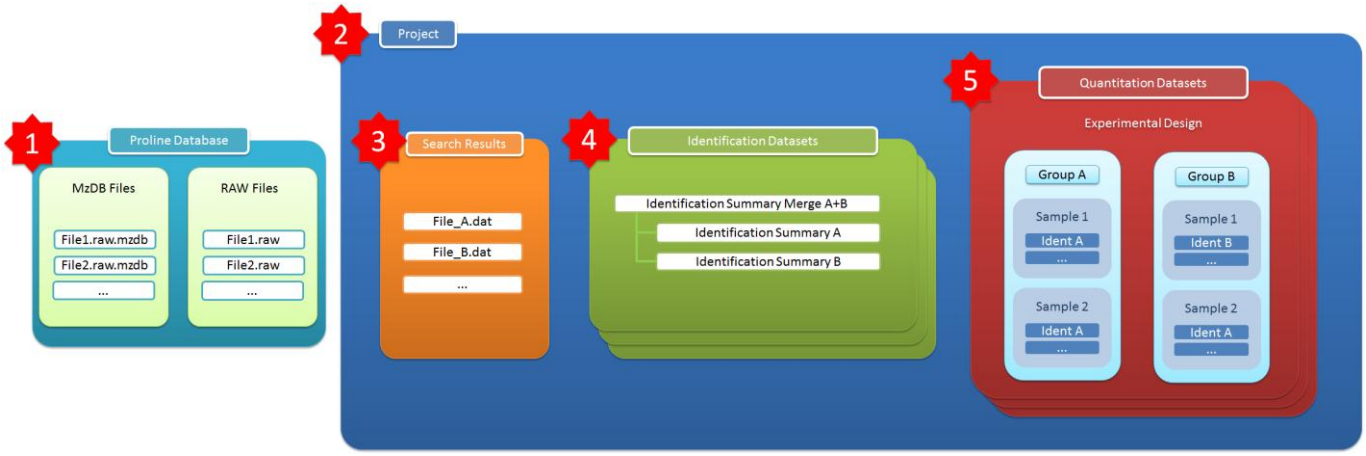
When exporting a whole Identification Summary in excel file, the following sheets may be generated (depending on options) :

- *search settings and infos* : Contains information on project and search settings parameters
- *import and filters* : Summary of used parameters during import, filtering and validation process
- *protein sets* : List of all Protein Set, valid or invalidated during Protein Sets Filtering. Some columns description :
    - *#sequences* (*#specific sequences*) : number of different peptide sequence identifying the Protein Set (specific : which does not identify any other valid Protein Set)

    - *#peptides* (*#peptides*) : number of different peptide (sequence + PTM) identifying the Protein Set (specific : which does not identify any other valid Protein Set)
    - *#peptide_matches* (*#specific_peptide_matches*) : number of different peptide spectrum matches identifying the Protein Set (specific : which does not identify any other valid Protein Set)
- *best PSM from protein sets* : List of best peptide spectrum matches (a single PSM per peptide is listed) for each Protein Set. Some columns description :
    - *#protein_sets* : number of Valid Protein Set identified by this PSM.
    - *#protein_matches* : number of Protein Match, which belong to at least 1 valid Protein Set, identified by this PSM.
    - *#databank_protein_matches* : number of Protein Match, validated or not, identified by this PSM. This is equivalent to the number of protein in fasta files containing the PSM.
- *protein matches in protein set* : list of Protein Matches in each Protein Set. A same Protein Match could thus appears few times if it belongs to different Protein Sets. (same column as protein set)
- *all PSMs from protein sets* : List of all peptide spectrum matches for each Protein Set. (same column as best PSM from protein sets)
- *statistics* : Some statistic values for the exported Identification Summary : number of Protein Set, modified peptides …

Getting Started : a Use case

Global Overview

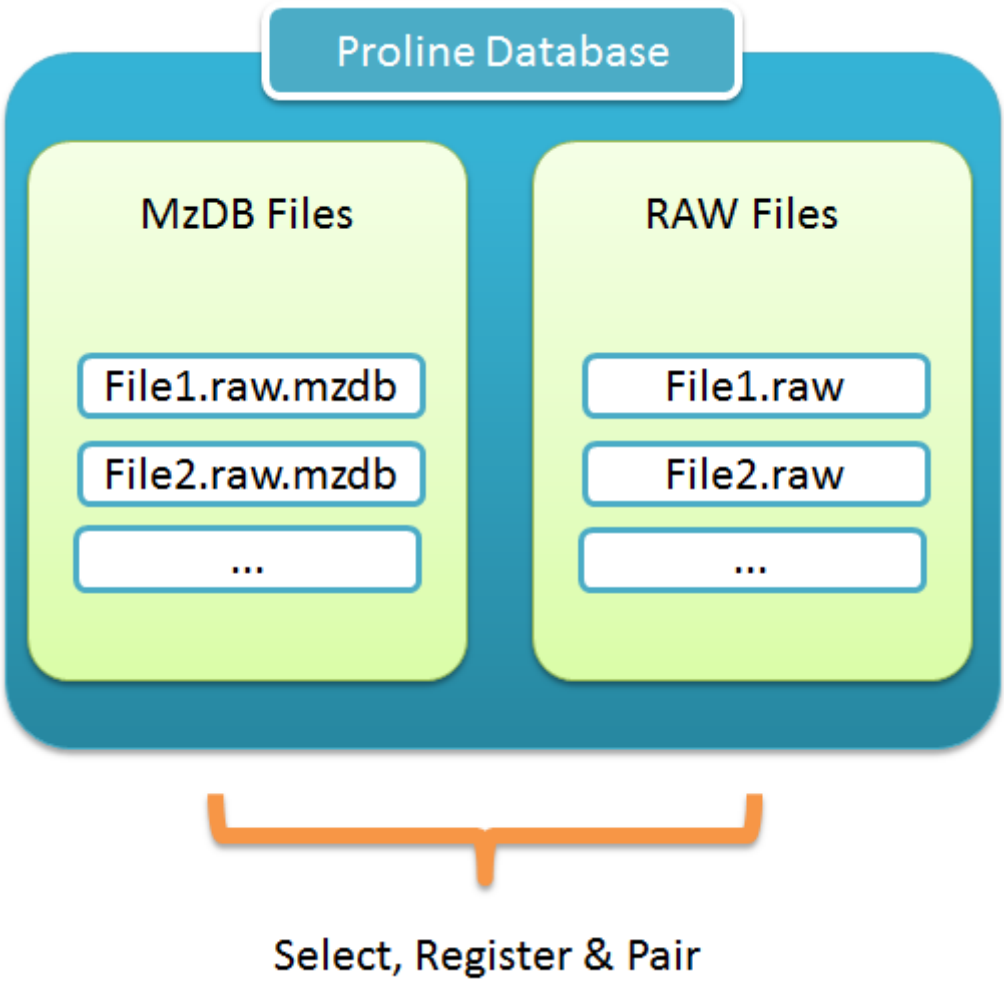


No matter which User Interface (Studio or Web) you will use, Proline's workflow remains the same:

1. If you plan to use the quantitation features of Proline, you'll need to register the Raw Files and their corresponding MzDB file into Proline's database in first place. This is an action you can perform aside from any project.

2. Once your files are ready to use, you must create a project. A project is attached to your user account, but you can “share it” with other users, so they can see it from their own account.
3. The first thing you need to do in your fresh new project is to import Result Files into it and associate them with the Raw Files you have registered, so that you will be able to create identification datasets you want to validate and quanitation experimental design based on your analyses.
4. Once your files are imported, you assemble them as datasets on which you will launch validation tasks with dedicated parameters
5. You can then launch a quantitation analysis by using your validated identification summaries, by re-creating you experimental, set up your own parameters and ratios.

Register and Pair Raw & MzDB Files



The Raw Files and their corresponding MzDB files are stored on your servers but they must be “registered” and paired into Proline's database.

See how to do this in Proline Web. In Proline Studio actually only MzDB files are taken into account. You specify thme during the quantitation process.

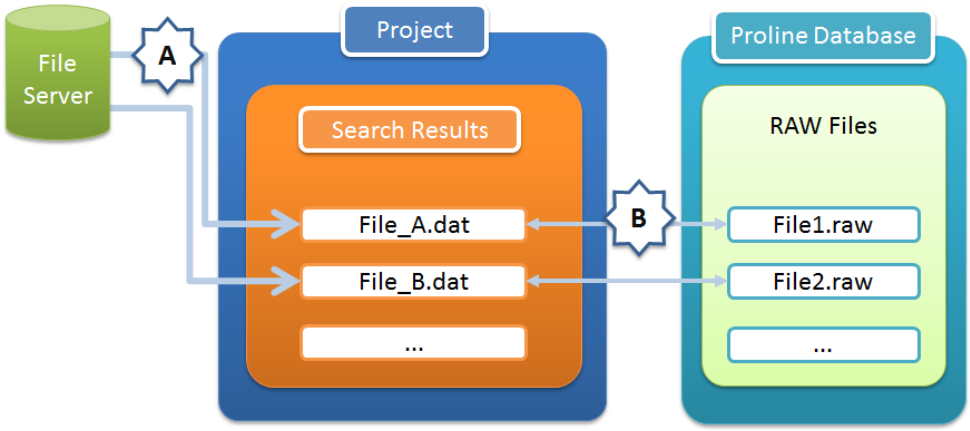
Create a Project

Create an Empty Project

A user can has many projects and share them with others. It's the place where you will import your Result Files (.dat, .omx), set up your validation and quantitation datasets

Read more about project creation in Proline Studio and Proline Web

Import Result Files

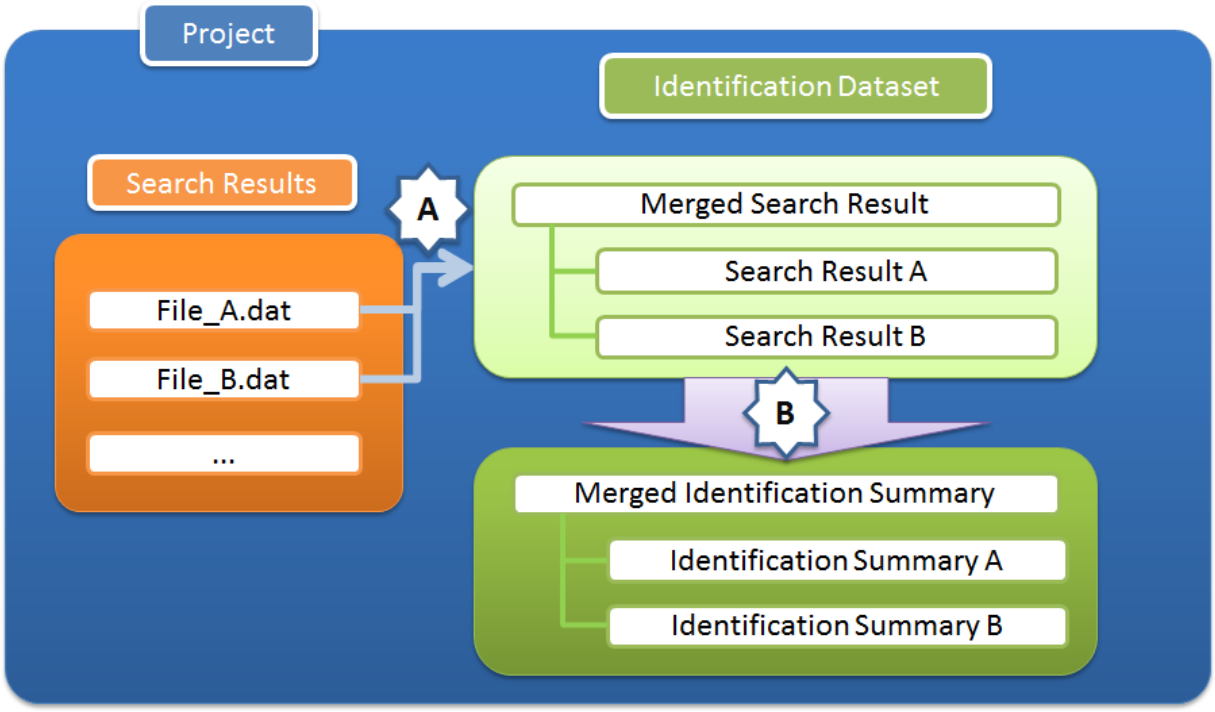


Import Result Files into Project and associate them with Raw Files

Once your project has been created, you'll need to import result files into it. This consists of storing your files data in Proline's databases. This is the first “task based” action will perform : Proline will run this action for a few minutes and you will be noticed when it's done.

See how to import files from the Proline Studio and the Proline Web interfaces.

Create & Validate Identification Datasets



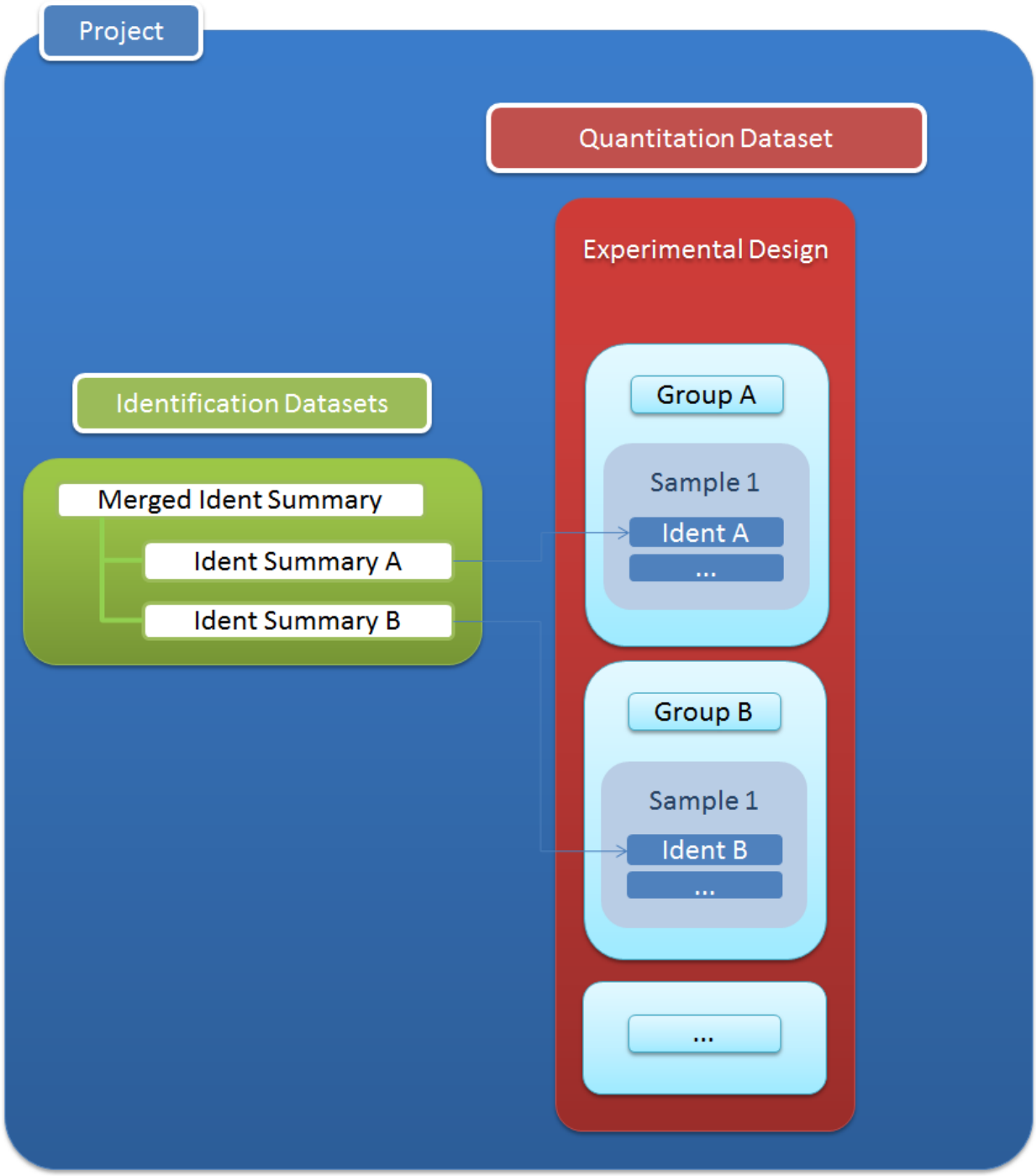
Create and Validate Search Results

Once your files have been imported to your project's database, you can use them as dataset on which you will perform validation operations. Datasets can be assembled under “aggregates” or treated as-is (in Proline Studio only). Once you've created a dataset with one or more files, you can launch a validation task on it, and then browse its result in what's called an Identifcation Validation Summary.

How to create a Dataset : in Proline Studio / in Proline Web

Hot to validate a Dataset : in Proline Studio / in Proline Web

Quantitation



Quantitations are built around your Experimental Design. You are able to recreate your technical and biological replicas hierarchy in Proline, set up your extraction parameters, define the ratios of your analysis and then launch the task.

See how it's done in Proline Studio and Proline Web

PostgreSQL optmization

PostgreSQL 9.1.x documentation (English) :
http://www.postgresql.org/docs/9.1/interactive/runtime-config-resource.html#RUNTIME-CONFIG-RESOURCE-MEMORY

Main configuration file is `postgresql.conf` located in PostgreSQL instance data directory.

Most usefull tunable parameters are (9):

Following recommended memory sizes are given for a server with **16 GiB** of physical memory and about **8 GiB** dedicated for the PostgreSQL instance.

- `max_connections` : Number of concurrent SQL sessions (each Proline Server task can use 1 to 5 SQL sessions, each Proline-Studio instance can use some SQL sessions).

Default value 100

- `tcp_keepalives_idle` : Number of seconds before sending a keepalive packet on an otherwise idle TCP connection. Help with broken router / firewall and checking for dead peers.

Default value 0 (2 hours) → 300 (5 minutes)

- `shared_buffers` Use about 1/4 of physical memory dedicated to the PostgreSQL instance.

Default value 32MB → 2048MB

- `checkpoint_segments` = Use (`shared_buffers` / 16) ; max. 64 or 256 for write-heavy bulk loading.

Default value 3 → 128

- `checkpoint_completion_target` = 0.9 for high value of `checkpoint_segments`.

Default value 0.5 → 0.9

- `temp_buffers` **Per session** Used for temporary tables creation.

Default value 8MB → 512MB

- `work_mem` = **Several per session** Used for hashing, sorting and `IN` operator when processing queries.

Default value 1MB → 4MB to 64MB

- `maintenance_work_mem` Used for intial index creation and `VACUUM` operations.

Default value 16MB → 1024MB

- `effective_cache_size` Assumption about the effective size of the disk cache to optimize index use (Monitor physical memory allocated by system to disk cache operations).

Default value 128MB → 4096MB